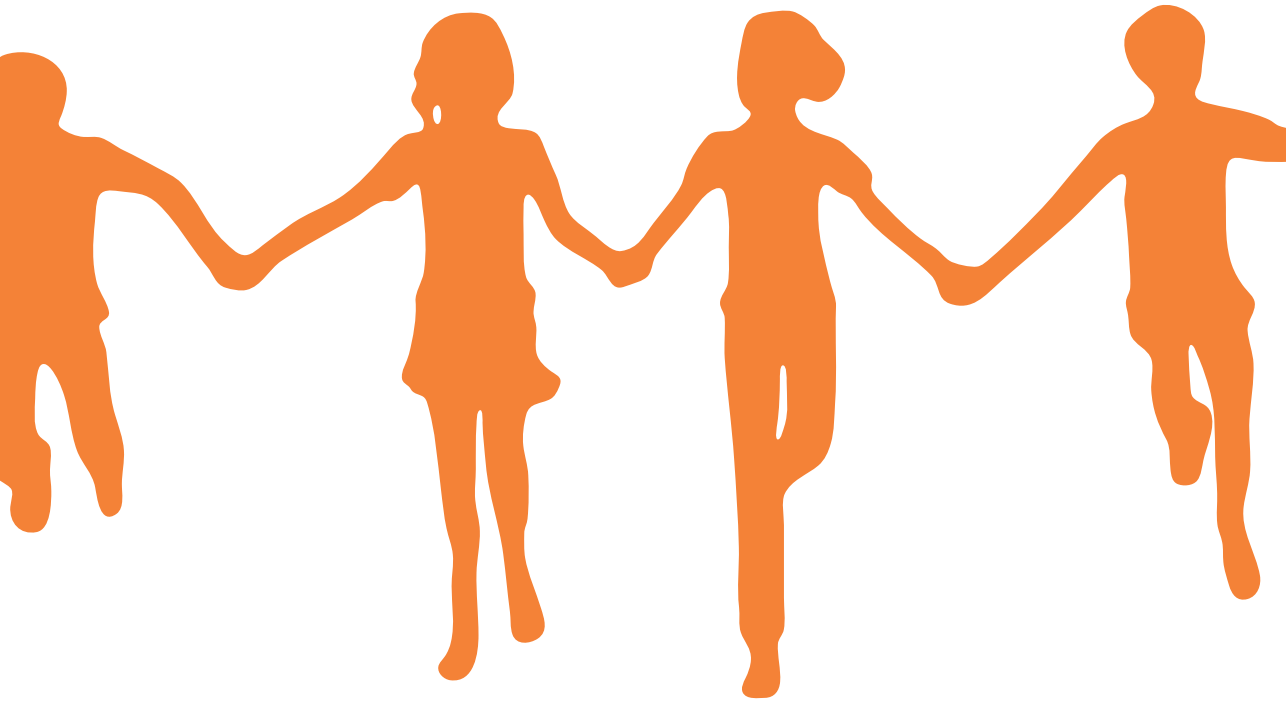
Restore the Balance

Side effects of dexamethasone in children with acute lymphoblastic leukemia



LIDEWIJ T. WARRIS

Restore The Balance
Side effects of dexamethasone in children with acute lymphoblastic leukemia

Lidewij Teresa Warris

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Restore The Balance
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Herstel de balans
Bijwerkingen van dexamethason in kinderen met acute lymfatische leukemie

Proefschrift

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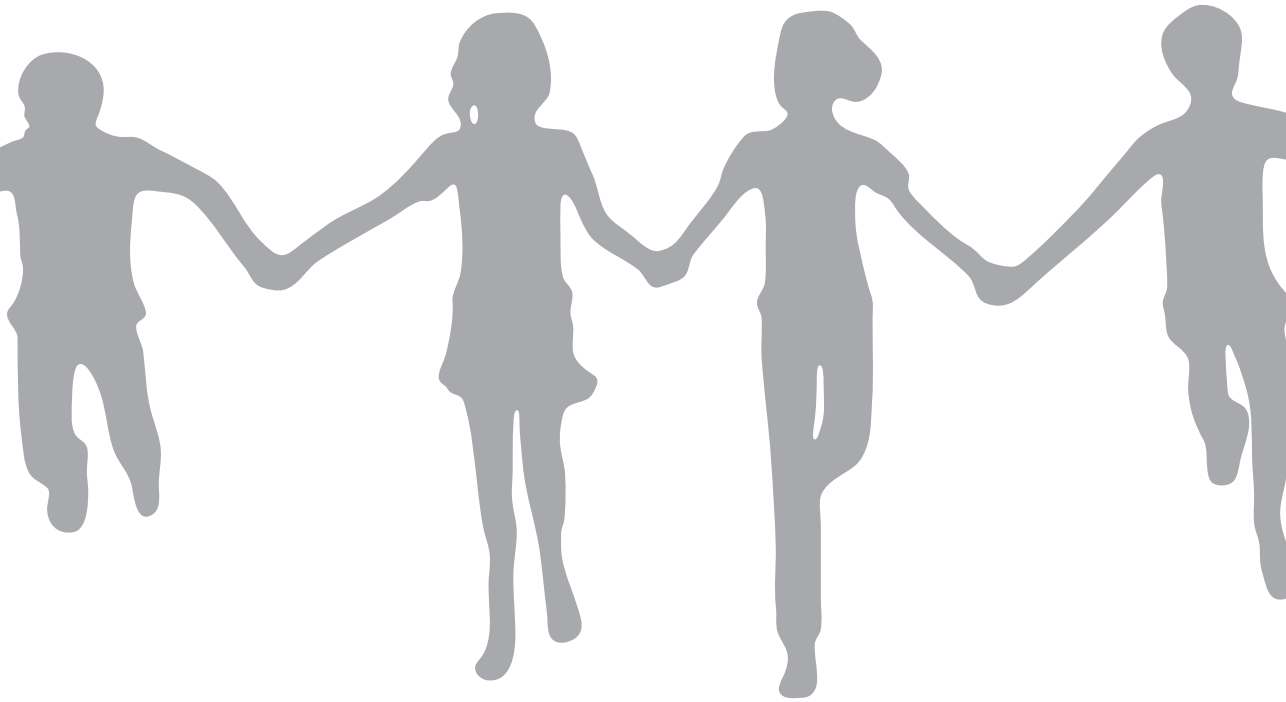
CONTENT

Chapter 1

General Introduction



Acute lymphoblastic leukemia

Acute lymphoblastic leukemia (ALL) is the most frequently occurring pediatric cancer, representing 120 new cases yearly in the Netherlands.[1] The peak incidence of this disease is between the ages of two and five years. In normal hematopoiesis the self-renewing hematopoietic stem cells in the bone marrow develop towards all different mature blood cell types (Figure 1). ALL is characterized by the presence of rapidly proliferating, monoclonal lymphoid progenitor cells. The uncontrolled expansion of leukemic cells leads to a compromised production of healthy mature blood cells, resulting in depletion of erythrocytes, platelets, and functional white blood cells.[2]

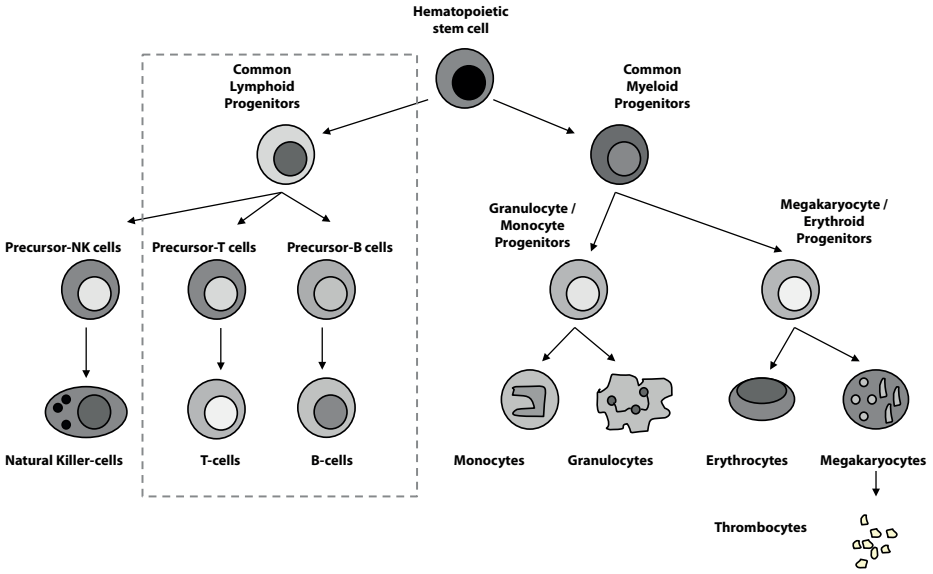


Figure 1. Normal hematopoiesis.

In children, ALL originating from immature B-cells is far more common (~85%) than ALL originating from immature T lymphocytes (~15%).[3]

Increased ALL survival

During the last decades, survival rates of childhood ALL have increased considerably (Figure 2) to a current overall five-year event-free survival of more than 85%.[4-6]

This increased survival is the result of the development of effective combination chemotherapy, optimized stratification of therapy, as well as optimized supportive care regimens. As outcome of ALL in children is good, attention is more and more focused on recognizing determinants of direct and late side effects in order to avoid unnecessary therapy-related morbidity and mortality.

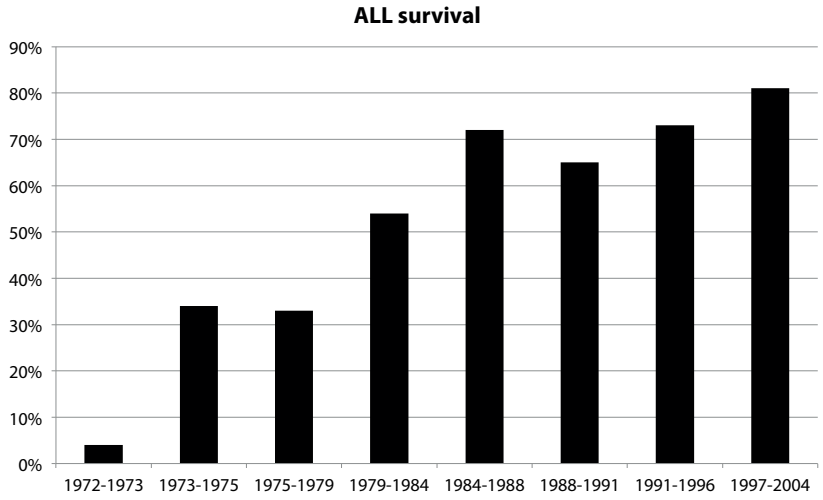


Figure 2. Increased ALL survival over the last decades (Pieters, NTVG 2010)

Treatment of ALL typically consists of an induction, consolidation, intensification, and maintenance phase. After induction therapy intensity is based on response, which is measured by the amount of remaining leukemic cells, also known as minimal residual disease (MRD).[7] In the Netherlands, currently, the Dutch Childhood Oncology Group ALL-11 protocol is used, that stratifies children with ALL towards standard-, medium- and high-risk regimens.

The maintenance phase of the medium-risk regimen plays a central role in this thesis. This phase of therapy is meant to eliminate MRD, and to prevent relapses, and consists of weekly methotrexate, daily mercaptopurine, vincristine, and dexamethasone therapy. Dexamethasone is administered in five-day courses in cycles of three weeks. (Figure 3)

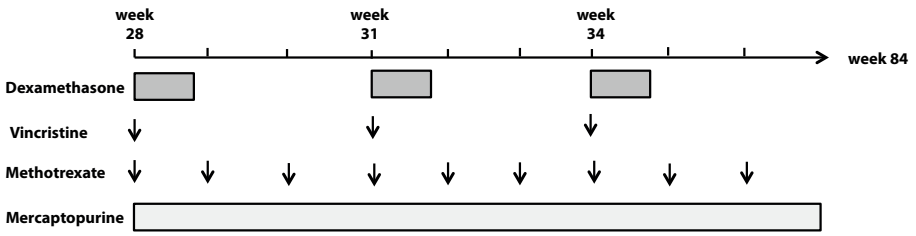


Figure 3. Detail of the maintenance phase of the current Dutch Childhood Oncology Group ALL-11 protocol, after discontinuation of asparaginase and anthracyclines.

Glucocorticoids

Prednisolone and dexamethasone are important components of ALL treatment. Together, they are referred to as glucocorticoids, because of their role in regulation of glucose levels, their synthesis in the adrenal cortex, and their steroidal structure. Apart from being one of the most valuable anti-leukemic agents, glucocorticoids also have anti-inflammatory and immunosuppressive effects.[8] In the current Dutch ALL medium risk protocol patients receive dexamethasone at least for 1,5 years during the maintenance phase. Dexamethasone pulses have been shown to be highly effective and are more potent in the treatment of ALL as compared to prednisone, due to its central nervous system penetration[9] and higher event free survival.[10-13]

A drawback of the treatment with glucocorticoids is the occurrence of serious side effects[11, 14] on metabolism (like hyperglycemia, hypertension, hyperlipidemia), body composition, bones and the brain. (Figure 4) Cerebral side effects, or neuropsychological side effects, include mood disorders, behavioral problems, cognitive effects, obsession with food, and sleeping disorders.[15, 16] Based on previous studies, these neuropsychological side effects occur in 5-75% of pediatric ALL patients treated with glucocorticoids. This in-

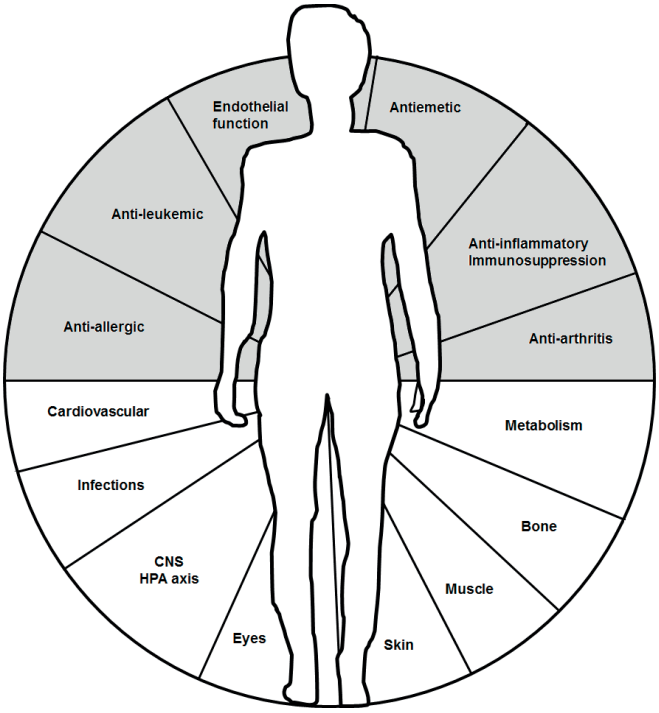


Figure 4. Effects of glucocorticoids. Upper half of circle (grey): therapeutic effects. Lower half of circle: side effects.

troduces a considerable impact on the quality of life during the long period of treatment in young children.[17] Because of the higher anti-leukemic activity and the higher potency to cause metabolic side effects[11, 14], it is conceivable that dexamethasone induces more neuropsychological side effects than prednisone.

Pathophysiology of dexamethasone related-neuropsychological side effects

Two receptors are important for the binding of glucocorticoids: the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR). Recent data emphasize that MRs in the brain play an important role in the regulation of mood, behavior, cognition and sleep.[18, 19] The GR and MR are expressed in the same order in the human hippocampus, and many other limbic brain regions.[20] Based on the high affinity of the MR for cortisol, it may be the predominant receptor during the circadian trough of cortisol secretion, while at circadian peak levels the MR and GR mediated actions are in balance and determine the stress responsiveness of an individual.

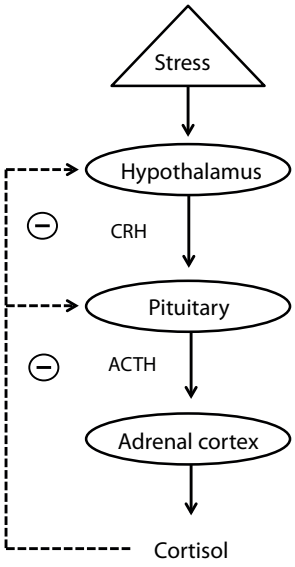


Figure 5. Schematic overview of the hypothalamic-pituitary-adrenal axis.

Disturbance of this GR:MR balance, for instance during dexamethasone treatment, can deregulate the stress system and enhance vulnerability to stress-related disorders.[21] Dexamethasone has a 30-40 fold higher potency to activate the GR than cortisol, and, in contrast to prednisolone, dexamethasone does not bind to the MR.[22] Dexamethasone suppresses the endogenous production of cortisol in the adrenals by its suppressive effect on the hypothalamus-pituitary-adrenal axis. (Figure 5) Therefore, in dexamethasone treated patients, the cerebral MRs are not occupied because the production of cortisol, which binds the cerebral MRs in healthy subjects, is fully suppressed.[22]

In contrast, prednisolone binds the GR, but has a low affinity for the MR. Prednisolone has an effect on the GR and the MR with a ratio of 5:1, so there is still some MR activation during prednisone treatment.[22] This characteristic binding affinity explains why neuropsychological side effects may be more severe during dexamethasone treatment compared to prednisone treatment. Treatment with dexamethasone pulses suppresses the pulsatile secretion of endogenous cortisol. During repeated exposure to exogenous glucocorticoids, the MR:GR balance may change, and thereby affecting mood, behavior, cognition and sleep.[23]

Several studies have shown that MR antagonism or cortisol depletion of the MR can cause serious neuropsychological side effects. (Figure 6a) MR knock-out mice showed increased anxiety behavior.[19] In addition, in rodent studies, MR antagonists have potent effects on behavioral responsiveness and coping styles.[18] In humans, treatment with MR antagonists has been associated with impaired selective attention, impaired recall of visual-spatial memory and diminished slow wave sleep.[19] Conversely, MR agonism had beneficial effects as add-on to anti-depressive treatment[24], and a gain of function haplotype of the human MR gene is associated with resilience against depression.[25]

The differential effects of natural and synthetic glucocorticoids on the brain caused by different binding properties to the GR and MR are also illustrated by examples from patients. Addison patients, who lack endogenous cortisol, have been found to experience mood disorders and impairment of cognitive function. They were found to have a better performance on cognitive function tests when treated with dexamethasone combined with cortisol, compared to dexamethasone only.[26]

Side effect directed interventions

Efficacy studies on interventions for dexamethasone-induced neuropsychological side effects are mainly limited to case reports. Only Pelletier *et al*, reported that both chlorpromazine and lorazepam were effective at minimizing glucocorticoid-related symptoms in a series of ten pediatric ALL patients.[27]

The various case reports about therapeutic options[10, 15, 17, 28-32] are limited to symptomatic treatment of mood and behavioral side effects. For example mood stabilizers, antidepressants, electroconvulsive therapy[31] and reduction or switch of steroids[17], have been suggested to be effective in the acute treatment of corticosteroid induced psychiatric symptoms.[15] Risperidone has been suggested to be effective for treatment of agitation, but the onset of mood-stabilizing and antipsychotic effects may take up to two weeks.[28, 32] In individual cases citalopram[30], antipsychotic promethazine[29] and potassium chloride supplements[10] were reported to be effective in case of behavioral side effects. Since therapeutic options for dexamethasone-induced neuropsychological side effects are scarce, the aim of this thesis was to explore a new therapeutic option, with a low patient burden, to diminish these side effects.

Hypothesis

The previous studies in mice and human led to our hypothesis that in children with ALL who receive dexamethasone treatment, cortisol depletion of the MR in the brain occurs, stronger than in prednisolone-treated patients, thereby causing exacerbation of serious neuropsychological side effects.[18, 19] (Figure 6b) Hence, we hypothesized that these side effects could be ameliorated by adding a physiological dose of the natural occurring hormone cortisol (hydrocortisone) that stimulates the MR in the brain in a

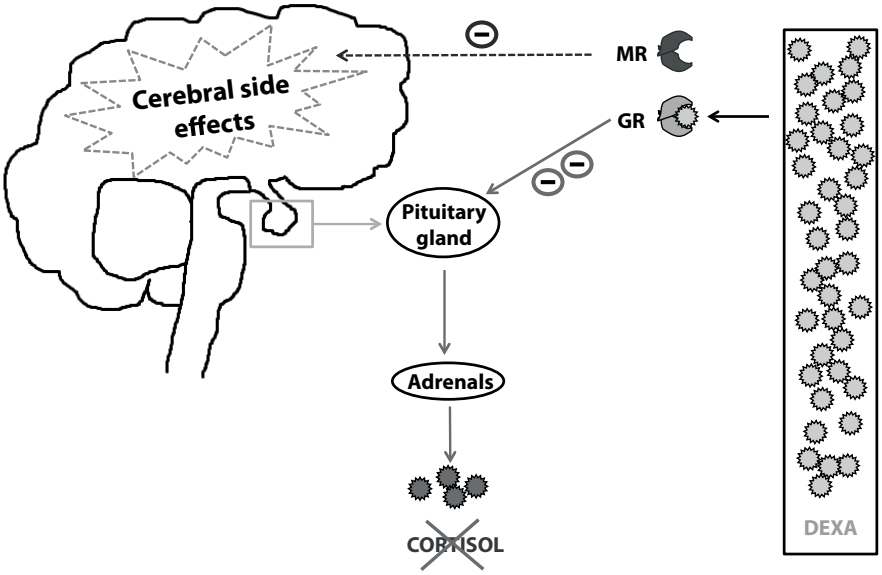


Figure 6a. Dexamethasone-treated children with leukemia. GR: glucocorticoid receptor. MR: mineralocorticoid receptor.

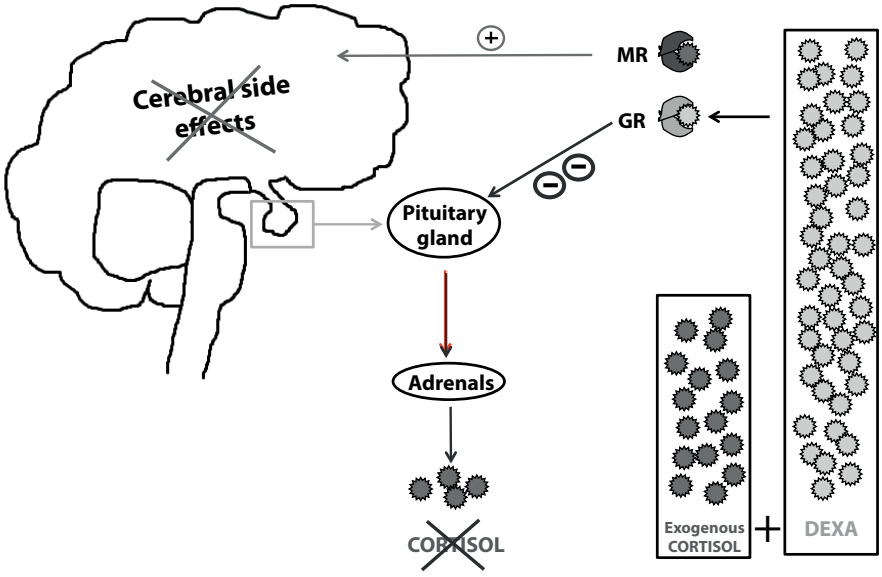


Figure 6b. New hypothesis. GR: glucocorticoid receptor. MR: mineralocorticoid receptor.

physiological way. An absolute prerequisite is that MR activation by this intervention does not interfere with efficacy of the pure glucocorticoids in treating ALL. It is conceivable that reduction of neuropsychological side effects of dexamethasone would lead to a major improvement in quality of life in children with ALL, which may also be true for patients with Addison's disease and rheumatologic diseases.

Study design

We tested our hypothesis with a double blind placebo-controlled randomized study with a crossover design. The multicenter study was performed in five pediatric oncology centers in the Netherlands, that all used one and the same protocol.

Scope of thesis

The aim of this study was threefold:

- The primary aim was to reduce dexamethasone-induced cerebral side effects on mood, behavior, sleep, and cognition by an intervention with physiological doses of cortisol.
- The secondary aim was to study whether dexamethasone-induced metabolic toxicity (i.e. hyperglycemia, insulin resistance, visceral fat gain, hypertension and hypercholesterolemia) occurred less frequently during intervention treatment compared to placebo.
- The third aim was to study the positive predictive value of a novel *in vivo* diagnostic test (salivary very low dose dexamethasone suppression test) on dexamethasone side effects.

Outline of this thesis

The first part of the thesis, **Chapter 2**, reviews the difference between the neuropsychological side effects of dexamethasone and prednisone in pediatric ALL patients. We hypothesized that neuropsychological side effects of dexamethasone could be decreased by addition of physiological doses of hydrocortisone during dexamethasone courses. Before we could pursue such a trial, we had to ensure safety in a preclinical study, which we describe in **Chapter 3**. This provided a safe background for our randomized controlled trial, the "Dexadagen study". The results of this trial are presented in **Chapter 4**. The results of the study on the acute effects of dexamethasone on components of the metabolic syndrome are presented in **Chapter 5**. To predict which patients may experience neuropsychological side effects of dexamethasone we studied the positive predictive value of the salivary very low dose dexamethasone suppression test, as described in **Chapter 6**. In **Chapter 7** we describe the typical exceptional dexamethasone-induced changes in eating behavior in a quantitative manner. Finally, we discuss the results of this thesis in **Chapter 8**, and an overall summary is provided in **Chapter 9**.

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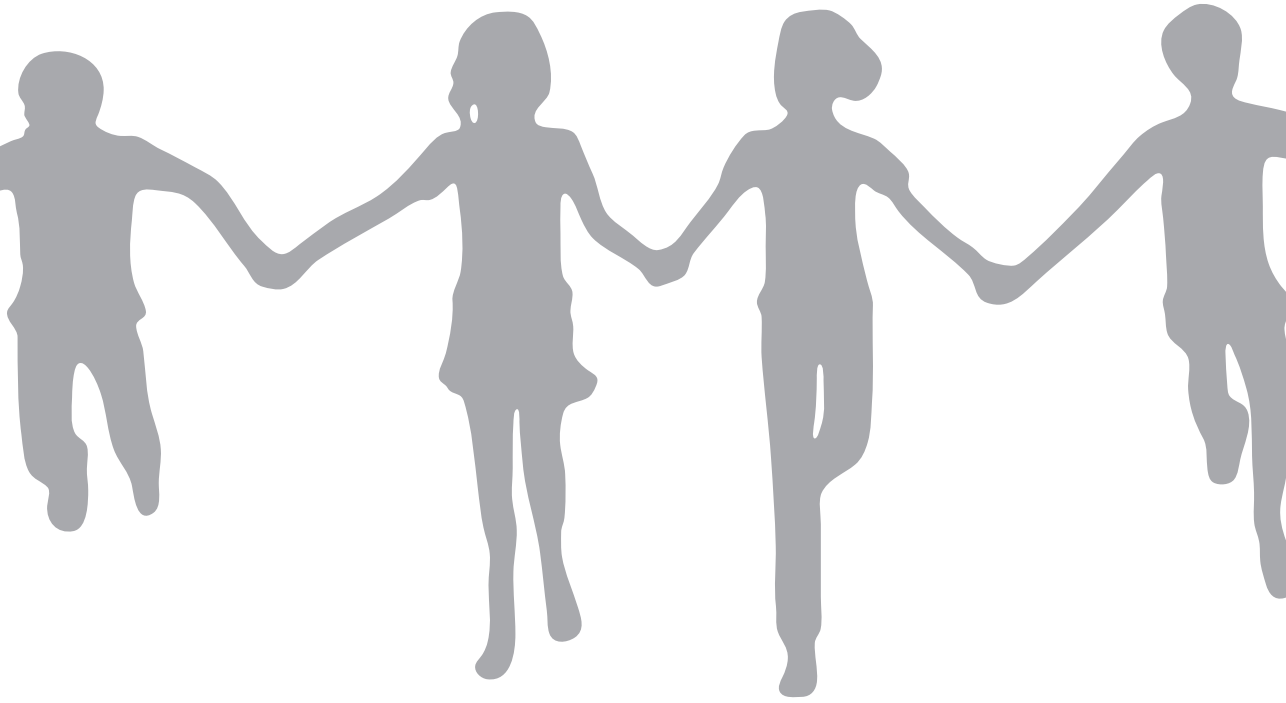
Chapter 2

Does dexamethasone induce more neuropsychological side effects than prednisone in pediatric acute lymphoblastic leukemia? A systematic review

Lidewij T. Warris, Marry M. van den Heuvel - Eibrink, Marissa A.H. den Hoed, Femke K. Aarsen, Rob Pieters, Erica L.T. van den Akker



Pediatric Blood & Cancer. 2014 Jul;61(7):1313-1318.

ABSTRACT

Steroid-induced neuropsychological side effects impact quality of life in children with acute lymphoblastic leukemia. Dexamethasone induces more metabolic side effects than prednisone. To evaluate whether dexamethasone also leads to more neuropsychological side effects, we reviewed all available literature. Randomized controlled trials with neuropsychological function as the primary or secondary outcome did not show clinically meaningful differences between dexamethasone and prednisone on cognition, mood or behavior.

INTRODUCTION

Corticosteroids, like prednisone and dexamethasone, are important in the treatment of acute lymphoblastic leukemia (ALL).[1] Dexamethasone is the preferred corticosteroid, because of the higher anti-leukemic activity and better central nervous system penetration than prednisone.[2-5] Beside the anti-leukemic effect, corticosteroids are known for their wide range of side effects. These include hyperlipidemia, hypertension and hyperglycemia, but also effects on body composition and bones.[4, 6] Early metabolic side effects are more apparent in dexamethasone-based than in prednisone-based schedules. Systemically, dexamethasone is about 18 times more potent than prednisone at suppressing short-term linear growth and stimulating weight gain, and about nine times more potent at suppressing bone turnover.[6] In addition, symptomatic osteonecrosis is an adverse event of dexamethasone in ALL treatment and has become a major cause of acute and long-term morbidity, particularly in adolescents.[7, 8] Dexamethasone also seems to be significantly more potent than prednisone in altering body mass index (BMI), insulin resistance[9] and causing hyperglycemia and myopathy.[2]

According to patients and their parents, the steroid-induced neuropsychological side effects are important negative determinants of the quality of life of children during cancer treatment.[10, 11] The risk of corticosteroid-related neuropsychological side effects is dependent on steroid dose and scheduling.[12] In view of the fact that pediatric ALL patients receive steroid pulses for a duration of approximately two years in most protocols, the steroid-related side effects on behavior, mood and cognition may potentially have a major impact on the child's daily life and development.

Because of the higher anti-leukemic activity and the higher potency to cause metabolic side effects, it is conceivable that more neuropsychological side effects, affecting behavior, mood and/or cognition, may occur with dexamethasone than with prednisone. However, previous reports seem to indicate conflicting outcomes on this point. Therefore we investigated the evidence by reviewing all published studies comparing neuropsychological side effects between dexamethasone and prednisone in children treated for ALL.

METHODS

The review included English-language studies of children, determined through a systematic search in the database of MEDLINE (1960 till December 2013), EMBASE (1960 till December 2013) and The Cochrane Library (till December 2013).

Search terms we used are (child*[tw] OR toddler*[tw] OR boy*[tw] OR girl*[tw] OR pediatr*[tw] OR paediatr*[tw] OR (adolescen*[tw] NOT adults[mesh])) AND

(Corticosteroid*[tw] OR Corticoid*[tw] OR dexamethason*[tw] OR prednisone*[tw] OR prednisolon*[tw]) AND (Mood Disorders[mesh] OR cognition[mesh] OR Cognition Disorders[mesh] OR behavior[mesh] OR Neuropsycholog*[tw] OR depress*[tw] OR affect*[tw] OR mood*[tw] OR cognit*[tw] OR behavio*[tw] OR fatigue*[tw] OR sleep*[tw] OR mania*[tw] OR panic*[tw] OR mental*[tw] OR eating behavior*[tw] OR eating disorder*[tw] OR food obsession* [tw] OR craving*[tw] OR hyperphagia*[tw] OR anxiety*[tw] OR attention*[tw] OR memory*[tw] OR executive function*[tw] OR processing speed*[tw]) AND (leukaemia*[tw] OR leukemi*[tw] OR ALL[tw]).

Articles were selected on the basis of title and abstract by two independent reviewers (LTW and MAHH) using the following inclusion criteria: (1) children with leukemia / ALL were receiving dexamethasone and/or prednisone; (2) neuropsychological side effects (mood, cognition, behavior, sleep) were compared between dexamethasone and prednisone; (3) original research; (4) written in English. We excluded case series (<10 subjects). All selected articles were assessed in full text by two reviewers to ensure eligibility. Discrepancies between reviewers during the review process were solved by discussion with the co-authors. In total 13 studies met the inclusion criteria (Figure 1). We evaluated eligible studies on study design, quality of the study, consistency and directness of the study by the Grading of Recommendations Assessment Development and Evaluation (GRADE) criteria of the GRADE working group.[13, 14]

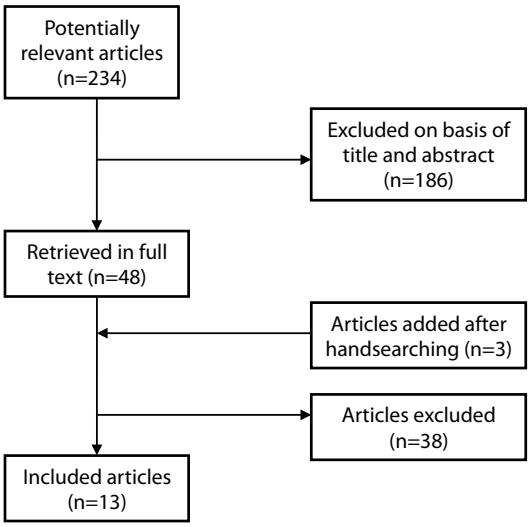


Figure 1. Selection of articles.

RESULTS

RCTs with neuropsychological side effects as primary endpoint

Five randomized controlled trials (RCTs) have compared neuropsychological side effects of dexamethasone and prednisone by a validated neuropsychological assessment in children with ALL as primary endpoint.[15-19] These trials have a high level of evidence, because of their study design without important limitations or inconsistencies. Three of the RCTs focused on the acute steroid-induced mood and behavioral changes[15, 16, 18], and two RCTs focus on long term neurocognitive[17, 19] as well as behavioral effects[17, 19]. All five

trials included steroid randomization, and used validated questionnaires or neurocognitive tests.

Behavior and mood. The three trials investigating acute steroid-related effects on mood and behavior had a different period of assessment with a range from induction therapy to one year from diagnosis. Despite these differences in period of assessment, the investigators showed consistent results and reported no significant difference in side effects on mood and behavior between patients treated with dexamethasone or prednisone (dexamethasone: prednisone dose ratio was considered 1:6-7). Mood and behavior was assessed during steroids by the parent-reported questionnaires; the Pediatric Quality of Life Inventory (PedsQL), the Child Behavior Checklist (CBCL) or the Behavior Rating Inventory of Executive Function (BRIEF).[15, 16, 18] The MRC UK ALL-99 study used the PedsQL that rates the child's physical, social and emotional function, at 3-6 months (T1) after diagnosis and one year (T2) after diagnosis in 45 mothers of a child with ALL.[15] The mean age of the children at T1 was 7.3 years. An increase in physical, social and emotional problems over the first year of therapy was shown with use of the PedsQL for both steroids, without significant differences between dexamethasone and prednisone.[15] The CBCL was used in the AIEOP-BFM study and the DFCI CP00-01 study to assess the child's behavior via parent-report.[16, 18] In the AIEOP-BFM study 12 of 37 ALL patients received dexamethasone during the first 28 days of treatment. 67% of children in the dexamethasone group showed more adverse psychological reactions after 4 weeks of continuous steroids, compared to 48% in the prednisone group, but this difference was not significant (OR=2.2, 95% CI: 0.5-9.1).[16] Side effects during continuation phase were measured in the DFCI CP00-01 study[18], where parents of 62 ALL patients reported behavior at baseline, after five days of steroids, and after one and two weeks off-steroids. They did not show a significant difference in behavioral side effects between the dexamethasone and prednisone treated group.[18] Interestingly, older children (≥6 years) treated with a standard risk protocol showed more problem behavior than those treated with the high-risk protocol, despite a threefold higher steroid dose for high-risk patients. The investigators hypothesized that more frequent delivery of intrathecal chemotherapy to standard risk patients (every 9 weeks vs. every 18 weeks) during the first six months of continuation therapy could potentially be contributory.[18]

Waber et al.[19] assessed long-term side effects on behavior and mood in the DFCI CP 00-01 protocol at a median time of 5.8 years post diagnosis, using parent-reported questionnaires; the BRIEF and the Behavioral Assessment System for Children-2 (BASC-2). They could not show a difference in behavioral and mood effects on the long term between patients treated with the two drugs.[19]

Cognition. Beside the trials on mood and behavior, two RCTs investigated the steroid-induced long-term neurocognitive side effects.[17, 19] Both trials had randomized steroid arms and used validated tests to assess neurocognitive function at a mean time of 9.8

years (CCG trial)[17] and a median time of 5.8 years (DFCI trial)[19] since diagnosis. The CCG trial investigated the long-term effect on intelligence, academic achievement, processing speed, concentration, memory and visual-motor integration in 92 childhood ALL survivors (mean: 3.3 years, range: 6-16 years).[17] They only found a minor negative effect of dexamethasone compared to prednisone on word reading (*P*=0.02), which was not clinically significant. The groups performed similarly on attention-concentration, visual-motor integration, numeric operations, spelling and memory. Also, there was no significant difference in the parents' report of neurological complications, psychotropic drug use, and need of special education services between dexamethasone and prednisone.[17]

The DFCI trial prospectively compared the difference in neurocognitive outcome between dexamethasone and prednisone in 170 survivors of ALL at a median time of 5.8 years post diagnosis by Wechsler tests.[19] The assessing psychologists were blinded for the steroid that was administered. Steroid type did not influence most of the outcomes. Only patients on the dexamethasone arm performed poorer on a measure of fluid reasoning (*P*=0.02). It is however important to stress that mean scores on nearly all outcomes in this study approximated the mean for the general population. A limitation of the DFCI trial is the inclusion of patients with cranial radiation in their treatment, so any differences attributed to corticosteroid exposure could in fact be confounded by radiation. They did not adjust for radiation dose and interaction between corticosteroids and radiation dose was not checked. The results of this trial generally confirmed the results of the CCG trial. In both studies the dexamethasone group used more special education, but differences were not significant (DFCI trial: dexamethasone 33% vs. prednisone 20%, *P*=0.09*)*.[17, 19]

RCTs with neuropsychological side effects as secondary endpoint

Behavior and mood. Beside the RCTs using neuropsychological side effects as primary endpoint, three large non-blinded trials randomizing prednisone versus dexamethasone studied acute neuropsychological side effects as secondary endpoint.[2, 4, 20] Unfortunately, no validated questionnaires were used to measure the effects on mood and behavior, because the primary outcome was event free survival[2, 4, 20] and central nervous system relapse[2]. This lack of standard validated tests degrades the level of evidence, despite the high quality study design. Fortunately, all studies measured side effects during similar phases of treatment, but they all lack a report on cognitive effects. The three RCTs consistently reported more acute side effects on mood and behavior in patients treated with dexamethasone compared to the prednisone group, although the difference was small and only significant in the MRC study[4], which was the only one that included patients > 9 years. Igarashi et al.[20] included ALL patients (n=359) under the age of 10 and found neuropsychiatric effects, such as severe agitation, in 1.7% of the children treated with dexamethasone during induction and intensification. No psychiatric side effects

were found in the prednisone arm of this study.[20] The CCG-1922 trial (n=1060) limited their steroid randomization and analysis to the induction phase, and no significant differences were found in neuropsychological side effects between both steroids.[2] The UK MRC ALL-97/99 trial (n=1603, 1-18 years) reported behavioral problems in 5.9% of the dexamethasone group during induction compared to 1.4% in the prednisone group (*P*=0.02).[4] The reported side effects varied widely, ranging from mood swings to severe depression and violence towards themselves or others. Three patients (0.4%) had a dexamethasone induced delusional psychosis.[4] Cessation of dexamethasone treatment led to rapid disappearance of symptoms. After cessation of steroids these patients were switched to prednisone with no reported significant recurrence of behavioral problems. No patients treated with prednisone developed severe mood changes that led to reduction of or discontinuation of steroids.[4]

Prospective observational studies

Behavior and mood. Two studies investigated side effects on mood and behavior of prednisone versus dexamethasone prospectively, but not in a randomized setting.[21, 22] These studies used validated questionnaires and psychological function was their primary outcome. Parent-reported CBCL and PedsQL questionnaires were used to assess behavioral and mood changes during treatment and two years post-therapy.[21, 22] Both studies found relatively more behavioral problems during dexamethasone compared to the prednisone group.[21, 22] According to Pound et al.[22] this difference was limited to older children (≥6 years). They investigated acute steroid-induced behavioral toxicity by parent report of 43 ALL patients with a mean age of 7 years. CBCL questionnaires were filled out after a 5-day steroid course and again after 2 or 3 weeks (off-steroids). They found significantly more total behavioral problems, affective problems and anxiety during dexamethasone compared to prednisone in the children above the age of five. Unfortunately, the number of patients treated with prednisone in this study was small (10 of 43), potentially influencing the findings.[22]

Marcoux et al.[21] used the CBCL at six time points during 4 years from diagnosis. The ALL patients had a mean age of 6 years at diagnosis. A trend for more externalized behavioral problems (disruptive, hyperactive, and aggressive behaviors) was observed during therapy in the dexamethasone group (*P*=0.057). However, the rate of externalized behavioral problems (20%) in the study population was relatively low.[21]

Cognition. Beside the two observational reports on mood and behavior, steroid-induced long-term effects on cognition were analyzed by three prospective non-randomized studies.[23-25] All studies used validated cognitive tests. The three studies differ in type of cognitive tests, steroid dose, and time of assessment. The observational study of Buizer et al.[25] assessed attentional function by the Amsterdam Neuropsychological Tasks (ANT) program in ALL patients at a mean time of 5.7 years after diagnosis.

Children treated with dexamethasone-based protocols (DCLSG ALL-6 and -9) did not differ in attentional function compared to children treated with prednisone-based protocols (DCLSG ALL-7 and -8). Interestingly, more subtle post-treatment attentional deficits were found in children that received intensified treatment. In view of the fact that the cumulative dose of intravenous methotrexate was the only treatment factor that was significantly higher in the ALL-intensified treatment group compared to the ALL-standard treatment group, these results suggest that the dosage of methotrexate is a factor in subtle post-treatment attentional deficits.[25] On the contrary, Waber et al.[24] and Edelmann et al.[23] showed a negative long-term effect of dexamethasone on cognition. The prospective DFCI study of Waber et al.[24] assessed cognitive function in ALL patients at a mean time of 4 years post diagnosis. The mean age of the patients at diagnosis was 3.7 years (DFCI 91-01: dexamethasone) and 5.3 years (DFCI 95-01: prednisone). They found significantly more neurocognitive impairment regarding measures of academic achievement (reading comprehension and arithmetic calculation), working memory, and learning disabilities in the dexamethasone group.[24] However, limitations of the study were the use of historic controls, the younger mean age of the dexamethasone group, which could possibly affect the outcome[17], and relatively higher rates of cranial radiation in the dexamethasone group (70% vs. 50% in the prednisone group, although not significant), which has a known effect on cognition. Very recently, Edelmann et al.[23] studied neurocognitive function in 38 adult survivors of childhood ALL of the TOTXIIIA (prednisone based) and TOTXIIIB (dexamethasone based) protocol with a median follow up time of 13 years (dexamethasone) and 16 years (prednisone) post diagnosis. They found lower performance in measures of short-term and long-term memory ($P<0.04$), intelligence ($P=0.03$), and academic tasks ($P<0.009$) in adult ALL survivors (range: 19-32 years) treated with dexamethasone.[23] The effect sizes were in the range of 3/4 to 1 standard deviation between groups.[23] They did not correct for follow-up time in the analyses. The results could be influenced by the variation in cumulative steroid dose between the subgroups of the St Jude trial. The patients in the TOTXIIIA protocol received 14% less steroids than the dexamethasone group.[23] Survivors in CCG study[17] were treated with less dexamethasone, compared to patients enrolled in the St Jude trial[23] and the prospective DFCI trial[24]. The subgroups in the TOTAL XIII study also differed significantly in the educational attainment, which may have been induced by the cognitive impairment of dexamethasone. However, no information on SES background was provided in this study. In the DFCI CP00-01 study[19] and the CCG study[17] patients treated with dexamethasone also needed more special education such as remedial teaching, compared to the prednisone group, but the difference between the dexamethasone and prednisone groups was not significant (Table I).

Table I. Included studies

Trial *Age at Dx, author*	N =	Study design	Assessment	Steroid dose	Treatment phase	Behavior and Mood	Cognition	Study limitation
MRC UK ALL-99 3-18 yrs (*Eiser, 2006*)	45	RCT	PedsQL	DEX: 6.5 mg/m^2/d PRED: 40 mg/m^2/d	3 mnths – 1 yr post Dx	ns	not tested	-
AIEOP-BFM ALL 2000 4-18 yrs (*Felder-Puig, 2007*)	37	RCT	CBCL	DEX: 10 mg/m^2/d PRED: 60 mg/m^2/d	Wk 1 – 19	ns	not tested	R only < 10 yrs
DFCI CP 00-01 1- 16 yrs (*Mrakotsky, 2011*)	62	RCT	CBCL, BRIEF	DEX: 6 mg/m^2/d PRED: 40 mg/m^2/d	Continuation	ns	not tested	-
CCG-1922 SR ALL, 6-16 yrs (*Kadan, 2009*)	92	RCT	WISC-IV, WIAT-II-A, CMS, CPT, Beery	R in Induction: DEX: 6 mg/m^2/d PRED: 40 mg/m^2/d	Mean 9.8 yrs post Dx (SD 0.5)	not tested	DEX: 1/3 SD lower on word reading (P=0.02)	-
DFCI CP 00-01 1-18 yrs *(Waber, 2013)*	170	RCT	WASI, WISC-IV, WAIS-III, ROCF, BASC-2, BRIEF, WJ-III, TML	DEX: 6 (SR + HR) / 18 (HR int) mg/m^2/d PRED: 40 (SR + HR)/ 120 (HR int) mg/m^2/d	4–9 yrs post Dx (median 5.8 yrs)	ns	DEX: lower score on fluid reasoning (P=0.02)	XRT subgroup
CCG-1922 SR ALL, 1-9 yrs (*Bostrom, 2003*)	1060	RCT	-	R in Induction: DEX: 6 mg/m^2/d PRED: 40 mg/m^2/d	Induction Continuation Maintenance	DEX: 1.1 % PRED: 0 %	not tested	No assessment
MRC ALL-97/99 SR/HR ALL, 1-18 yrs (*Mitchell, 2005*)	1603	RCT	-	DEX: 6.5 mg/m^2/d PRED: 40 mg/m^2/d	Induction Maintenance	DEX: 5.9 % PRED: 1.4% (P=0.02)	not tested	No assessment
Tokyo L95-14 SR/IR ALL, <10 yrs (*Igarashi, 2005*)	359	RCT	-	DEX: 8 (ind) / 6 (int) mg/m^2/d PRED: 60 (ind) / 40 (int) mg/m^2/d	Induction Intensification	DEX: 1.7% PRED: 0%	not tested	No assessment

Table I. Included studies (continued)

Trial *Age at Dx, author*	**N =**	**Study design**	**Assessment**	**Steroid dose**	**Treatment phase**	**Behavior and Mood**	**Cognition**	**Study limitation**
DFCI 2005-01, COG-0331, 0232 and 0434 SR/HR, 3-18 yrs *(Pound, 2012)*	43	Observational	CBCL, PedsQL	unknown	Maintenance	6-18 yrs: DEX >PRED: total + affective problems and anxiety 3-5 yrs: ns	not tested	Small subgroups: 76.7% dex 23.2% pred.
DFCI 91-01 or **DFCI 95-01** SR/HR, 0-17 yrs *(Marcoux, 2012)*	138	Observational	CBCL	DEX: 6 (SR) / 18 (HR) mg/m^2/d PRED: 40 (SR) / 120 (HR) mg/m^2/d	From Dx with 4yrs follow-up	DEX: trend for more externalized behavioral problems (P=0.057)	not tested	-
DFCI 91-01 (dex) **DFCI 87-01** (pred) SR/HR , 6-12 yrs *(Waber, 2000)*	67	Observational	WISC-R, WISC-III, ROCF	DEX: 6 (SR) / 18 (HR) mg/m^2/d PRED: 40 (SR) / 120 (HR) mg/m^2/d	Mean 4 yrs post Dx (SD 0.6)	not tested	DEX: worse on measures of academic achievement (P<0.02), working memory (P<0.03), and learning (P<0.02)	XRT subgroup
DCLSG ALL-6, ALL-7, ALL-8, ALL-9 SR/IR/HR, 4.9 yrs *(Buizer, 2005)*	36	Observational	ANT	DEX: 6 mg/m^2/d (ALL-6 and -9) PRED: 60 / 100 mg/m^2/d (ALL-7 and -8)	Mean 5.7 yrs post Dx (SD 3.4)	not tested	ns (0.25 < P< 0.99)	Small subgroups

Table I. Included studies (continued)

Trial *Age at Dx, author*	**N =**	**Study design**	**Assessment**	**Steroid dose**	**Treatment phase**	**Behavior and Mood**	**Cognition**	**Study limitation**
TOTXIIIA, TOTXIIIB 3-18 yrs *(Edelmann, 2013)*	38	Observational	WASI, WJ-III test of achievement, TML	Maintenance: DEX: 8 mg/m^2/d PRED: 40 mg/m^2/d	12-17 yrs post-Dx (median: 15.9 (pred) 13.3 (dex))	not tested	DEX: worse on measures of short-term and long-term memory (P<0.04), intelligence (P=0.03), and academic tasks (P<0.009)	Small sample size

Studies on dexamethasone (DEX) and prednisone (PRED) were graded (GRADE system) as high (light grey) or low (dark grey) level of evidence. Trials on long-term effects are grey. Various assessments were used: Child's Behaviour Checklist (CBCL), Behavioural Rating Inventory of Executive Function (BRIEF), Behavioural Assessment System for Children (BASC), Pediatric Quality of Life Inventory (PedsQL), Wechsler Intelligence Scale for Children- Fourth Edition (WISC-IV), Wechsler Individual Achievement Test–Second Edition–Abbreviated (WIAT-II-A), Beery Developmental Test of Visual Motor Integration (Beery), Conners' Continuous Performance Test II (CPT), Children's Memory Scale (CMS), Rey-Osterrieth Complex Figure Test (ROCF), Wechsler Abbreviated Scale of Intelligence (WASI), Woodstock-Johnson-III Test of Achievement (WJ-III test of achievement) , Test of Memory and Learning (TML), Amsterdam Neuropsychological Tasks computer-aided assessment program (ANT). Dx = diagnosis. XRT = cranial radiotherapy. R = randomization. ns = not significant.

DISCUSSION

Taking all trials into consideration, we found that half of the studies report more neuropsychological side effects with dexamethasone compared to prednisone, both on short-term as well as on long-term in children with ALL.[4, 21-24] However, if we focus on RCTs with neuropsychological outcome as primary endpoint, there is no significant difference in short-term and long-term effects on mood and behavior between both steroids[15, 16, 18, 19] and there is only little indication of a negative long-term effect on cognition of dexamethasone compared to prednisone in the absence of a clinically significant difference[17, 19]. In regard to the effectiveness and the ultimate aim of the treatment of childhood cancer, that is to say, with a view to the cure, this fact is reassuring, for dexamethasone clearly carries an advantage over prednisone by virtue of its greater beneficial potential.[2-5]

In order to accurately evaluate the acute steroid-related effects on mood and behavior based on the literature it is important to take a few problems into account. First, the discrepancy in acute effects on behavior and mood between the MRC trial[4] and some observational studies[21, 22, 24] on one hand and the three direct RCTs[15, 16, 18] on the other hand, which is primarily due to study design but could also be influenced by the lack of a subdivision in age groups in the RCTs.[15, 16, 18] It is conceivable that especially older children, as reported by Pound et al.[22], may have more side effects on dexamethasone compared to prednisone.

Furthermore, it is remarkable that clinicians report less[4] mood and behavioral side effects than parents[16] by validated tests. An underestimation of the frequency of side effects is possible in the RCT's with psychological side effects as secondary endpoint.[2, 4, 20] Another difficulty with assessment of steroid related mood and behavioral changes is the use of parent-reported questionnaires in all trials, which could deviate from the child's opinion. Unfortunately, assessment of behavior and mood with self-reported questionnaires was not performed, although all studies included older patients, so they could have used self-reported questionnaires.

To accurately assess the acute side effects on mood and behavior and compare the side effects between steroids, a double blind RCT would be required. Such a RCT may currently be difficult to pursue, because of the proven higher anti-leukemic activity of dexamethasone.[2] The results of the prospective studies and RCTs on mood and behavior show contradictory results. However, it is important to note that the prospective studies apart from their inferior study design, have either a small sample size or report relatively low behavioral problems. The unexpected finding of the DFCI study[18] about the influence of intrathecal chemotherapy frequency on behavioral problems will need further investigation.

In contrast to results of the trials on mood and behavior, the data on cognitive effects of steroids seem to be more in agreement. No study has compared differences of short-term cognitive effects between dexamethasone and prednisone. The prospective DFCI trial[24] and the recent SJCRH study[23] suggest that survivors of childhood ALL treated with dexamethasone are at higher risk for long-term memory problems 4 years[24] but also 16 years[23] post diagnosis. In contrast, the DCLSG study[25] reported no difference in attentional function 4 years post diagnosis comparing both steroids, but suggests that the cumulative dose of intravenous methotrexate is a factor in subtle post-treatment attentional deficits. The randomized trials[17, 19] on long-term cognitive function only show a subtle difference between dexamethasone and prednisone, limited to a minor decrease in word reading without clinical significance on dexamethasone in the CCG trial[17], and a minor decrease on a IQ measure of fluid reasoning on dexamethasone in the blinded DFCI RCT[19]. Mean scores on nearly all outcomes approximated the mean for the general population in the RCTs. The discrepancies between results of prospective studies and RCTs could be due to differences in time of follow-up, age at diagnosis, cumulative dose of glucocorticoid administered, or neurocognitive assessment procedures.[23] An extensive St. Jude Lifetime Cohort Study demonstrated that in adult survivors of childhood ALL previous use of dexamethasone was associated with significant neurocognitive impairment (on attention and executive function) after a median follow-up time of 26 years since diagnosis, adjusting for methotrexate exposure.[26] Buizer et al.[25] did not find an association between previous dexamethasone exposure and attentional dysfunction six years after diagnosis (at a mean age of 11 years). The differences in elapsed time since diagnosis and neurocognitive tests could contribute to the different outcomes. However, the findings of the St. Jude Lifetime Cohort Study underline the importance of further investigation of the effects of both steroids on long-term neurocognitive function.

Based on this review of the literature, we conclude that there is no high-level of evidence confirming a significant difference in short-term effects of dexamethasone and prednisone on behavior and mood in children with ALL. Moreover, there is no clinically significant difference in long-term cognitive effects between both steroids.

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Chapter 3

Hydrocortisone does not influence glucocorticoid sensitivity of acute lymphoblastic leukemia cells

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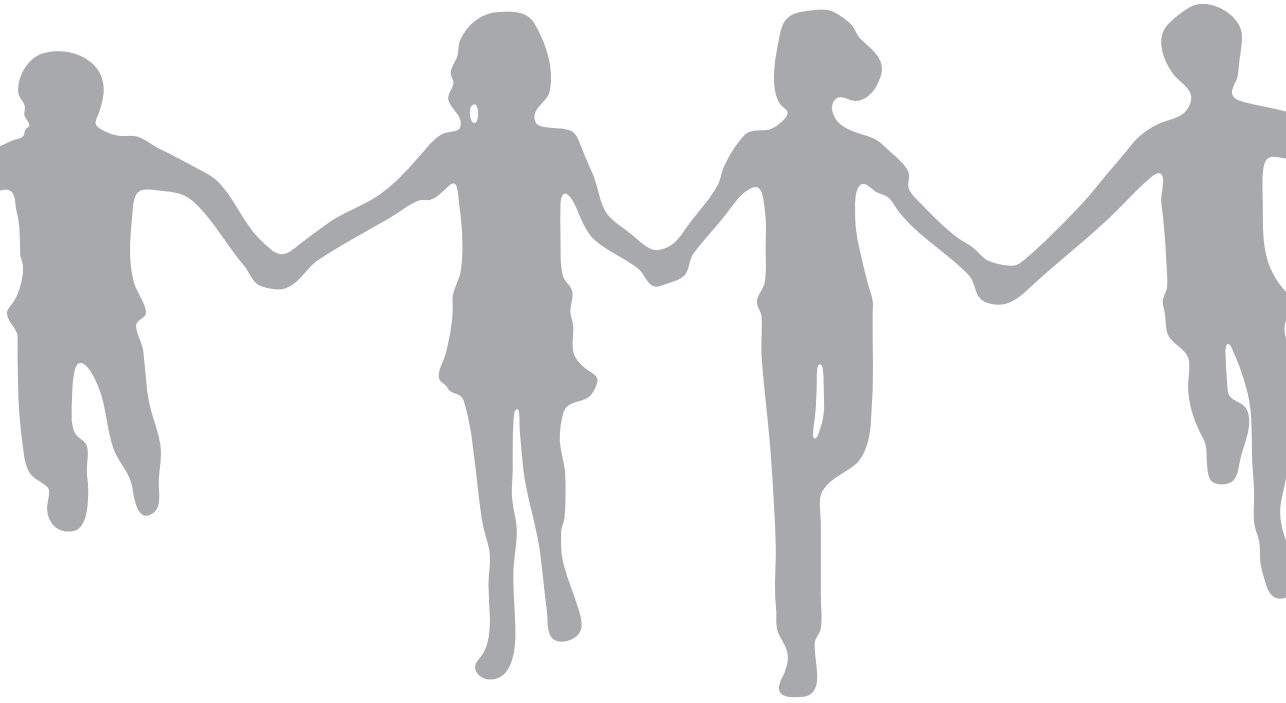

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Dexamethasone is the preferred glucocorticoid in the treatment of pediatric acute lymphoblastic leukemia (ALL), as its anti-leukemic activity *in vitro* is 7 to 16-fold higher than that of prednisolone[1] and it is associated with a higher event-free survival than prednisone.[2] However, serious neuropsychological side-effects have been described in 5-75% of children with ALL during treatment with dexamethasone, as reflected by obsession with food, sleeping disorders, and by mood, cognition and behavioral problems, sometimes even resulting in psychosis and depression.[3, 4] This may tremendously affect patients' quality of life during the two years of ALL treatment.[3]

These neuropsychological side effects may be caused by binding of dexamethasone to the glucocorticoid receptor (GR) expressed in brain tissues.[4] However, recent studies imply that the mineralocorticoid receptor (MR) is important in the regulation of mood, behavior and sleep.[5, 6] Cortisol can bind to both the MR and the GR, although cortisol has a 10-fold higher affinity for the MR.[5] In contrast, dexamethasone does not bind to the MR and its potency to activate the GR is 30- to 40-fold higher than that of cortisol.[7] Similarly, prednisolone has a higher binding-affinity (~5-fold) for GR compared to MR.[7] Recent data suggest that the MR in dexamethasone-treated patients is not fully saturated by endogenous cortisol.[7] This is caused by a reduced production of cortisol due to the fact that dexamethasone triggers a negative feedback-loop affecting the hypothalamus-pituitary-adrenal axis.[7] Studies in animals and small case series of patients with a depression suggest that a reduced level of cortisol has serious effects on mood, behavior and sleep.[5, 6] Based on these findings we hypothesize that dexamethasone-induced depletion of cortisol in the brain may cause or exacerbate the neuropsychological side-effects in children suffering from ALL. It is therefore feasible that administration of hydrocortisone (i.e. the naturally occurring cortisol now used as medicine) may reduce the neuropsychological side effects associated with dexamethasone treatment by circumventing this negative feedback-loop through direct activation of MR-mediated signaling as visualized in Supplemental Figure S1. To enable the clinical application of such an intervention strategy, however, an absolute prerequisite is that MR activation does not interfere with the anti-leukemic efficacy of glucocorticoids (dexamethasone and prednisolone). To this aim, we examined the MR and GR levels and the effect of hydrocortisone on the cytotoxicity induced by dexamethasone and prednisolone in leukemic cell lines and freshly obtained ALL cells of children with newly diagnosed ALL.

The mRNA levels of MR and GR expressed in leukemic patients' cells were estimated by probeset 205259_at for MR and 232431_at for GR from a previously published set of gene expression data generated using Affymetrix U133 plus 2.0 gene arrays.[8, 9] These levels were confirmed by RTqPCR (see supplemental methods). The genetic subtype of each patient was determined by means of FISH, RT-PCR and by utilizing a 110-probeset gene expression signature which enables the classification of ALL in (cytogenetic) subtypes.[8] A methyl-thiazol-tetrazolium salt drug cytotoxicity assay (MTT-assay) was used to select

cases being either *in vitro* highly sensitive, intermediate resistant or highly resistant to prednisolone, using exactly the same cut-off levels as previously reported.[9] These lethal concentrations for 50% of the cells (LC50) have been shown to be predictive for clinical outcome of children with newly diagnosed ALL.[9-11]

The mRNA levels of MR and GR did not differ between glucocorticoid sensitive, intermediate and resistant patients' ALL cells. Interestingly, the *ETV6-RUNX1*$^{+}$ subtype expressed higher MR mRNA levels than the other ALL subtypes ($p \leq 0.001$), although expression levels were still relatively low (Figure 1A). RT-qPCR analysis of MR and GR levels indicated that the mRNA levels obtained by both methods were strongly correlated for MR ($R=0.88$, $p<0.0001$ (Supplemental Figure S2A) and moderately for GR ($R=0.48$, $p=0.02$ (Supplemental Figure S2B). RT-qPCR also confirmed that *ETV6-RUNX1+* cells have higher MR mRNA levels than other subtypes of ALL ($p \leq 0.001$) (Supplemental Figure S2A). GR mRNA levels were higher compared to MR mRNA levels, in both microarray and RT-qPCR analyses (Figure 1C).

In order to study whether hydrocortisone can be safely combined with dexamethasone in the treatment of ALL, we first tested hydrocortisone and corticosteroids in four

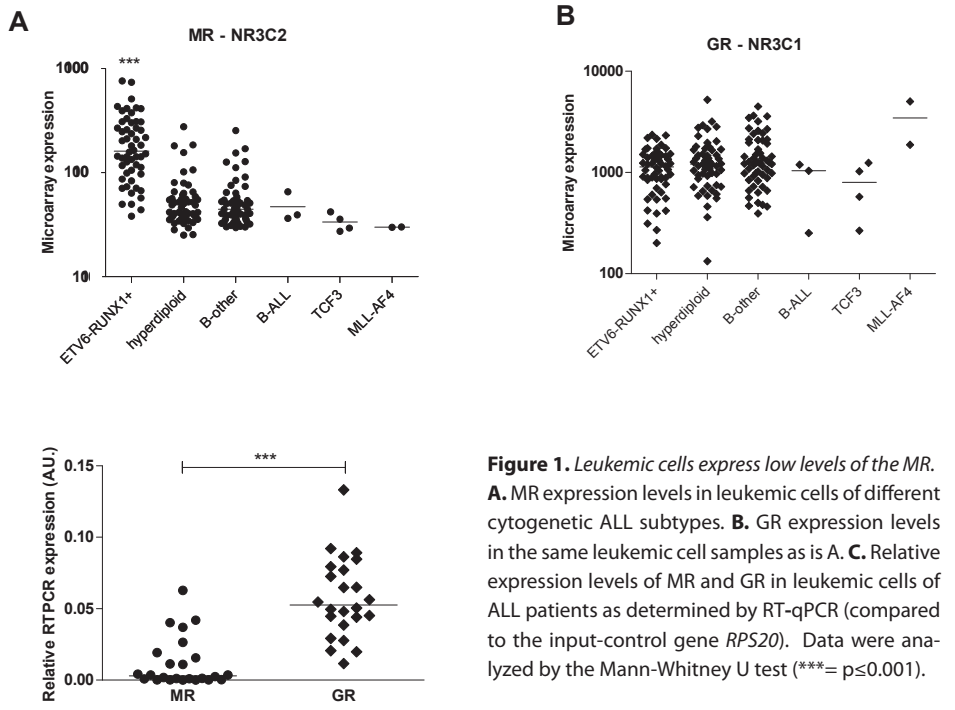

Figure 1. *Leukemic cells express low levels of the MR.* **A.** MR expression levels in leukemic cells of different cytogenetic ALL subtypes. **B.** GR expression levels in the same leukemic cell samples as is A. **C.** Relative expression levels of MR and GR in leukemic cells of ALL patients as determined by RT-qPCR (compared to the input-control gene *RPS20*). Data were analyzed by the Mann-Whitney U test (***= $p \leq 0.001$).

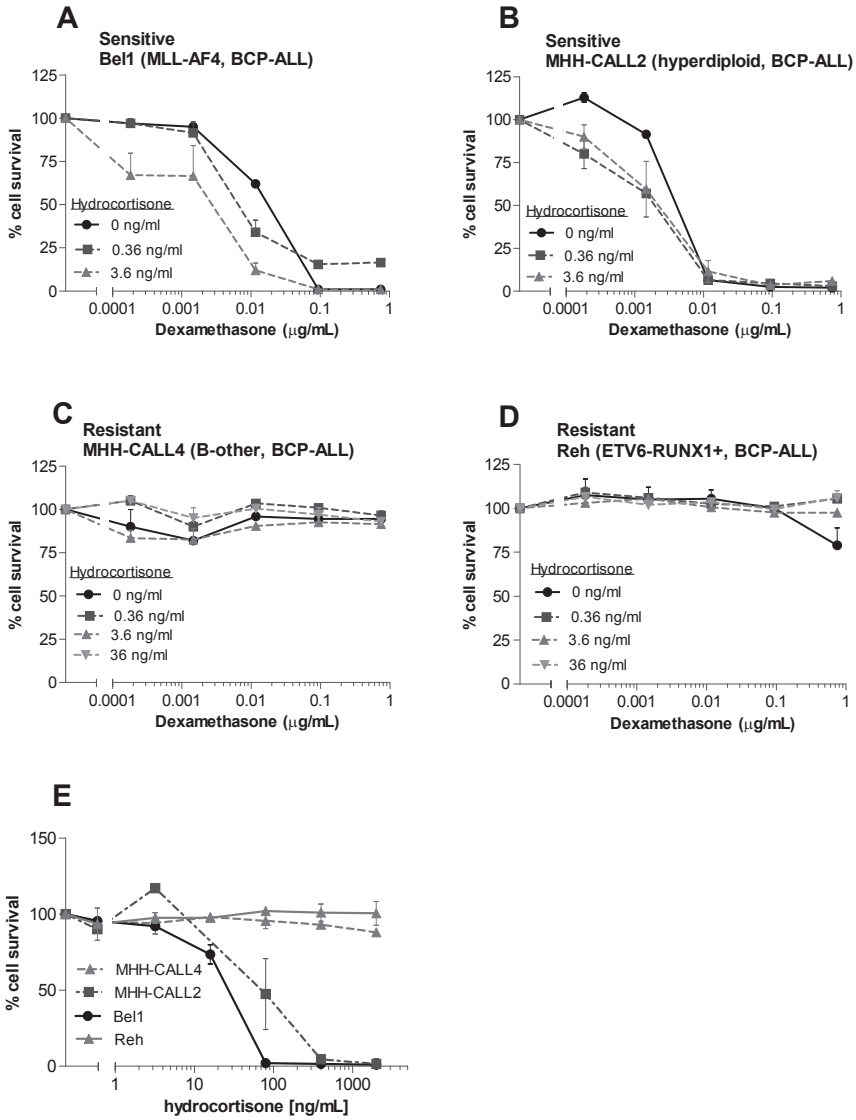

Figure 2. *Hydrocortisone does not induce resistance to glucocorticoids in leukemic cell lines*.
A. Cytotoxicity of hydrocortisone in combination with dexamethasone in a glucocorticoid sensitive Bel1 cell line. **B.** Cytotoxicity of hydrocortisone in combination with dexamethasone in a glucocorticoid sensitive MHH-CALL2 cell line. **C.** Cytotoxicity of hydrocortisone in combination with dexamethasone in a glucocorticoid resistant MHH-CALL4 cell line. **D.** Cytotoxicity of hydrocortisone in combination with dexamethasone in a glucocorticoid resistant Reh cell line. **E.** Hydrocortisone dose-response curve of glucocorticoid sensitive (Bel1, MHH-CALL2) and 2 glucocorticoid resistant (MHH-CALL4, Reh) cell lines.
Responsiveness of leukemic cell lines was determined by an 4-day total cell kill MTT-assay. The experiment was done twice for each of the 4 depicted cell lines. Sensitivity to dexamethasone was corrected for cell death induced by hydrocortisone as single agent to determine the synergistic or antagonistic effect of the drug combination. Data are presented as mean plus SEM.

leukemic cell lines purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany): Bel1 (*MLL-AF4*$^{+}$, BCP-ALL), MHH-CALL2 (hyperdiploid, BCP-ALL), MHH-CALL4 (B-other, BCP-ALL), Reh (*ETV6-RUNX1*$^{+}$, BCP-ALL). An MTT-assay was used using previously reported conditions.[9-11] MTT assays are suitable to test the sensitivity of leukemic cells for prednisolone and dexamethasone and these results are indicative for the prognosis of children with newly diagnosed ALL.[1, 9, 10] MTT assays measure total cell kill, but do not reveal the underlying mechanism of cell death. We previously showed that prednisolone induces apoptosis in leukemic cells of patients as illustrated by a decrease in mitochondrial membrane potential, increased caspase 3 cleavage and Annexin V positivity / propidium iodide negativity.[12] Hydrocortisone (Bufa Pharmaceutical Products) induced cell death in the glucocorticoid sensitive cell lines Bel1 and MHH-CALL2, whereas it did not affect the viability of glucocorticoid resistant cell lines MHH-CALL4 and Reh (Figure 2E). In combination, hydrocortisone did not affect or even sensitized glucocorticoid sensitive cell lines to dexamethasone (Sigma) or prednisolone (Bufa Pharmaceutical Products; Figure 2A-B and Figure S3C), whereas the level of resistance remained unaffected in resistant cell lines upon increasing concentrations of hydrocortisone (Figure 2C-D and Figure S3A-B).

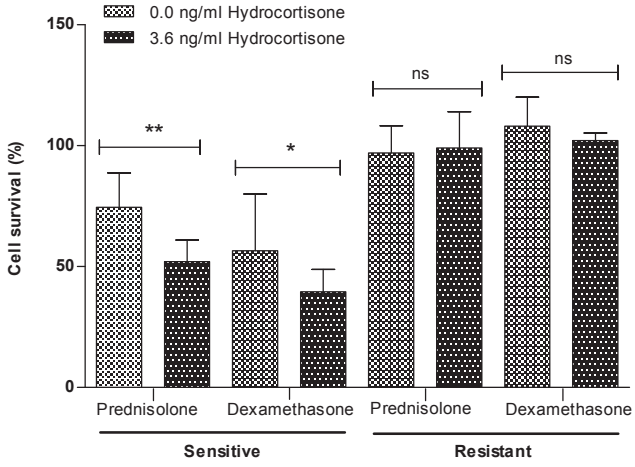

Figure 3. *Hydrocortisone does not induce resistance to glucocorticoids in patients' leukemic cells taken at initial diagnosis.*

An MTT-assay was used to determine the cytotoxic effect of hydrocortisone (3.6 ng/mL) in combination with dexamethasone (0.012 µg/mL) or prednisolone (0.061 µg/mL for sensitive patients and 0.49 µg/mL for resistant patients) in patients' leukemic cells. Patients' cells of different ALL subtypes were included (*ETV6-RUNX1*$^{+}$ B-ALL, B-other, hyperdiploid B-ALL, T-ALL). Statistical analysis was performed with a Mann-Whitney U test (**= $p \leq 0.01$, *= $p \leq 0.05$, ns = not significant). Data are presented as median and interquartile range. Sensitivity was corrected for cell death induced by hydrocortisone as single agent to determine the synergistic or antagonistic effect of the drug combinations. Further details on the drug sensitivity of different ALL subtypes are described in the supplemental documents.

Subsequently, we aimed to confirm these results in primary patients' cells of different ALL subtypes (*ETV6-RUNX1*$^{+}$, B-other, hyperdiploid and T-ALL). Hydrocortisone sensitized glucocorticoid sensitive patients' cells to dexamethasone or prednisolone (Figure 3), including *ETV6-RUNX1*$^{+}$ cells (Supplemental Figure S4-S5). Hydrocortisone did not affect the level of resistance to glucocorticoids of resistant patients' leukemic cells. (Figure 3)

Our in vitro studies show that addition of hydrocortisone does not interfere or even sensitizes ALL cells to glucocorticoids. The (sensitizing) effect of hydrocortisone is found for both types of glucocorticoids tested, i.e. dexamethasone and prednisolone. The expression level of the receptor for hydrocortisone, i.e. MR, is very low compared to the expression levels found for GR in leukemic patients' cells. The MR levels were remarkable high in *ETV6-RUNX1*$^{+}$ leukemic cells compared to other subtypes of ALL, for which an explanation is yet lacking. The higher MR levels in *ETV6-RUNX1*$^{+}$ patients' leukemic cells did not affect the response to hydrocortisone and glucocorticoids.

In conclusion, addition of hydrocortisone does not interfere with the response of leukemic cells to dexamethasone and prednisolone. Our next aim is to determine whether dexamethasone-induced neuropsychological side effects can be prevented by co-administration of hydrocortisone. To this aim, we have initiated a double-blinded randomized control trial in which we test the effect of physiological dosages of hydrocortisone during dexamethasone treatment on neuropsychological symptoms in children with newly diagnosed ALL (NTR3280).

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SUPPLEMENTAL FIGURES

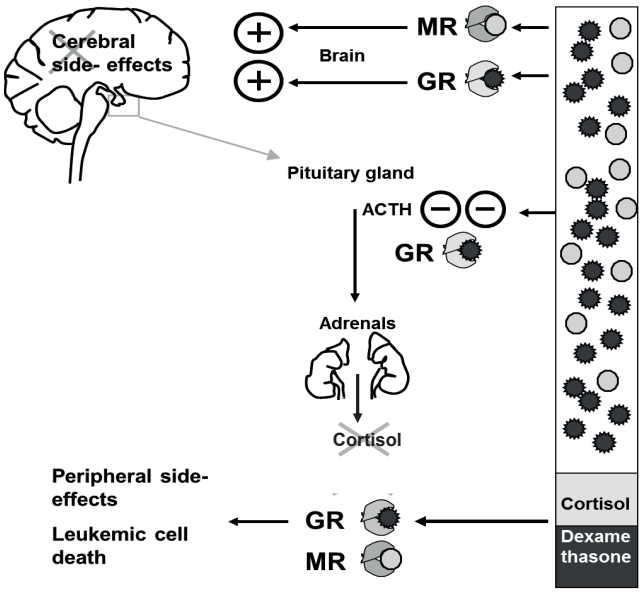

Figure S1. *Hypothesis.*
Dexamethasone treatment is known to deplete endogenous cortisol levels. This may cause the cerebral side-effects reported for this drug. Exogenous applied cortisol (hydrocortisone) may reduce these side-effects through binding to the mineralocorticoid receptor for which dexamethasone has no affinity. GR = glucocorticoid receptor, MR = mineralocorticoid receptor.

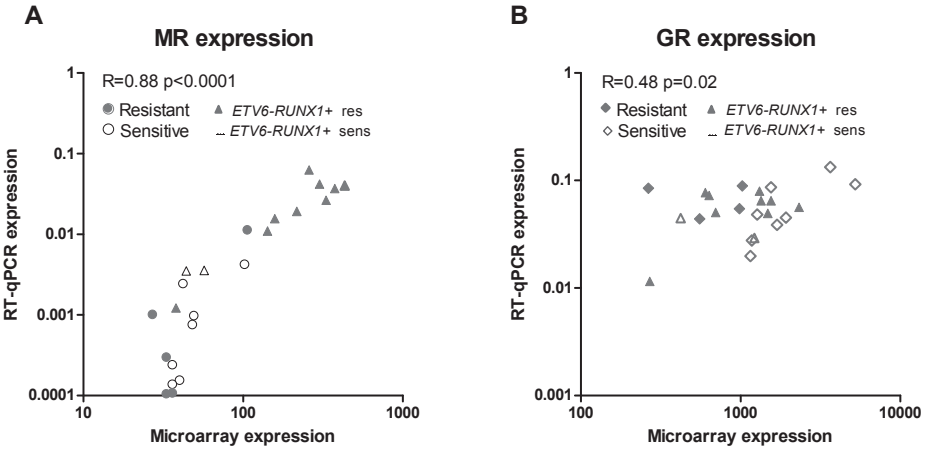

Figure S2. *Correlation between microarray and RT-qPCR determined expression levels of MR and GR.*
A. Correlation between microarray and RT-qPCR of MR, R=0.88, p<0.0001. B. Correlation between microarray and RT-qPCR of GR, R=0.48, p=0.02. MR = mineralocorticoid receptor, GR = glucocorticoid receptor. Spearman's rank correlation coefficient was calculated to compare microarray gene expression results to RT-qPCR results.

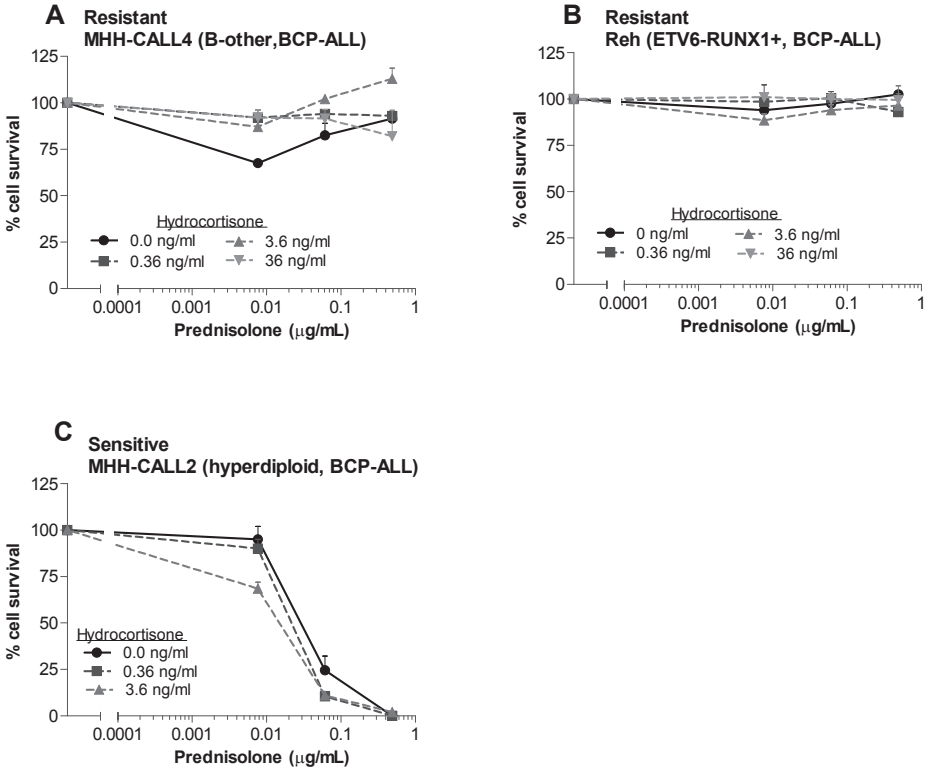

Figure S3. *Hydrocortisone does not induce resistance to prednisolone in leukemic cell lines*.

A. Cytotoxicity of hydrocortisone in combination with prednisolone in a glucocorticoid resistant MHH-CALL4 cell line. **B.** Cytotoxicity of hydrocortisone in combination with prednisolone in a glucocorticoid resistant Reh cell line. **C.** Cytotoxicity of hydrocortisone in combination with prednisolone in a glucocorticoid sensitive MHH-CALL2 cell line.

Responsiveness of leukemic cell lines was determined by an MTT-assay. Sensitivity to prednisolone was corrected for cell death induced by hydrocortisone as single agent to determine the synergistic or antagonistic effect of the drug combination.

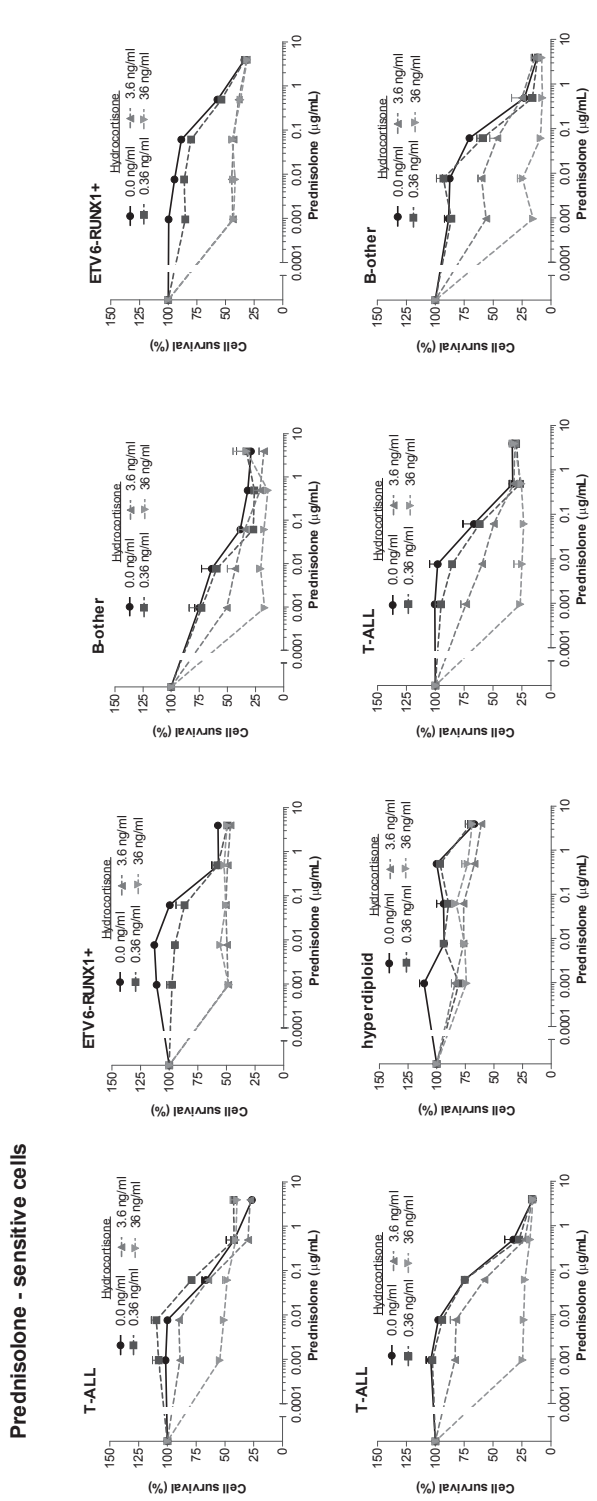

Figure S4. *Hydrocortisone sensitizes leukemic patients' cells of different subtypes which are intrinsic sensitive to prednisolone.*
Effect of hydrocortisone (0 ng/ml, 0.36 ng/ml, 3.6 ng/ml, 36 ng/ml) on prednisolone sensitivity of glucocorticoid sensitive primary patients' cells (hyperdiploid, *ETV6-RUNX1+*, T-ALL, B-other). Data are presented as mean plus SEM. Responsiveness of leukemic cells was determined by an MTT-assay. The cell survival of prednisolone-exposed cells was corrected for cell death induced by hydrocortisone to determine the sensitizing effect of hydrocortisone.

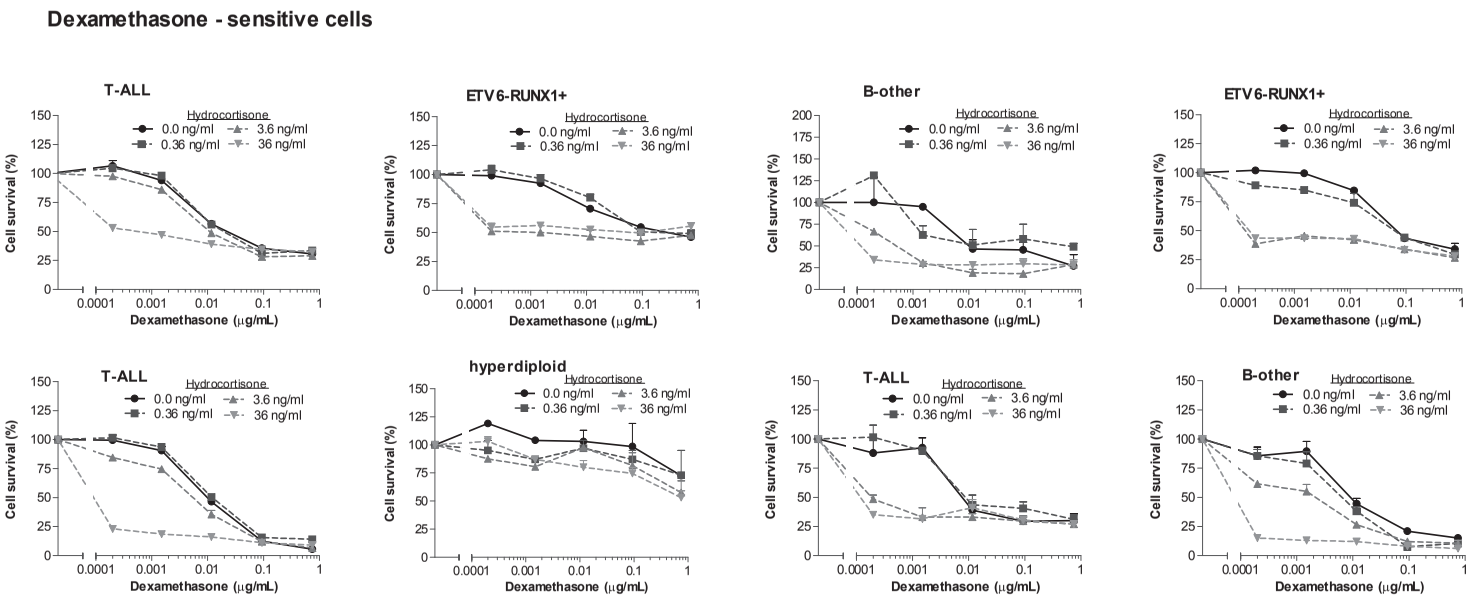

Figure S5. *Hydrocortisone sensitizes leukemic patients' cells of different subtypes which are intrinsic sensitive to dexamethasone.*
Effect of hydrocortisone (0 ng/ml, 0.36 ng/ml, 3.6 ng/ml, 36 ng/ml) on dexamethasone sensitivity of glucocorticoid sensitive primary patients' cells (hyperdiploid, ETV6-RUNX1+, T-ALL, B-other). Data are presented as mean plus SEM. Responsiveness of leukemic cells was determined by an MTT-assay. The cell survival of dexamethasone-exposed cells was corrected for cell death induced by hydrocortisone to determine the sensitizing effect of hydrocortisone.

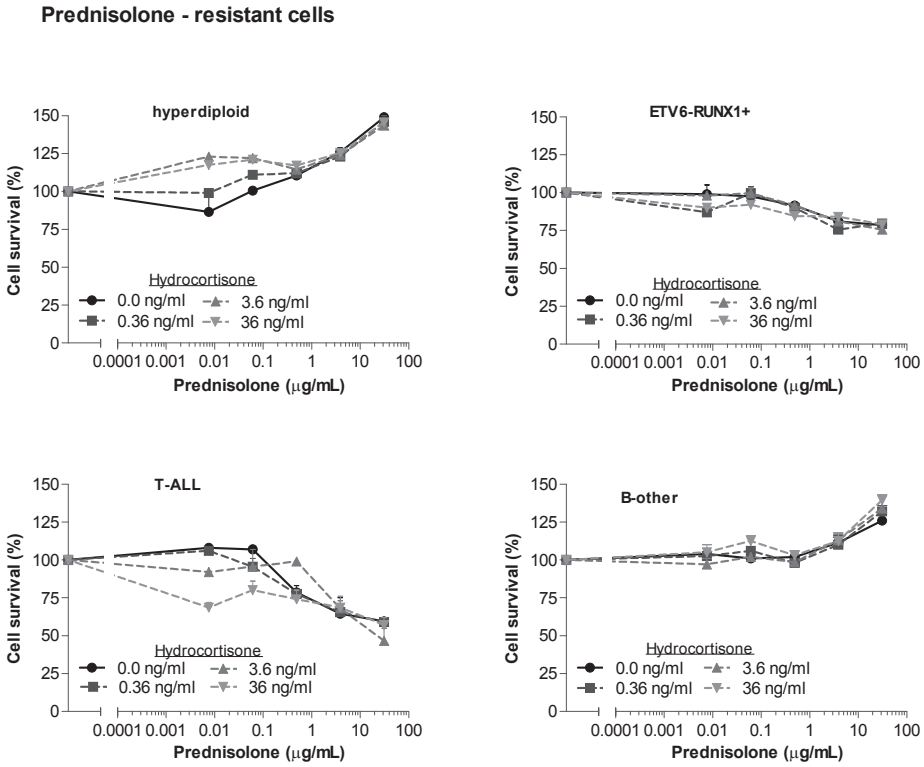

Figure S6. *Hydrocortisone does not induce more resistance to leukemic patients' cells which are intrinsic resistant to prednisolone in different subtypes of ALL.*

Effect of hydrocortisone (0 ng/ml, 0.36 ng/ml, 3.6 ng/ml, 36 ng/ml) on prednisolone sensitivity of glucocorticoid resistant primary patients' cells (hyperdiploid, ETV6-RUNX1+, T-ALL, B-other). Data are presented as mean plus SEM. Responsiveness of leukemic cells was determined by an MTT-assay. The cell survival of prednisolone-exposed cells was corrected for cell death induced by hydrocortisone to determine the sensitizing effect of hydrocortisone.

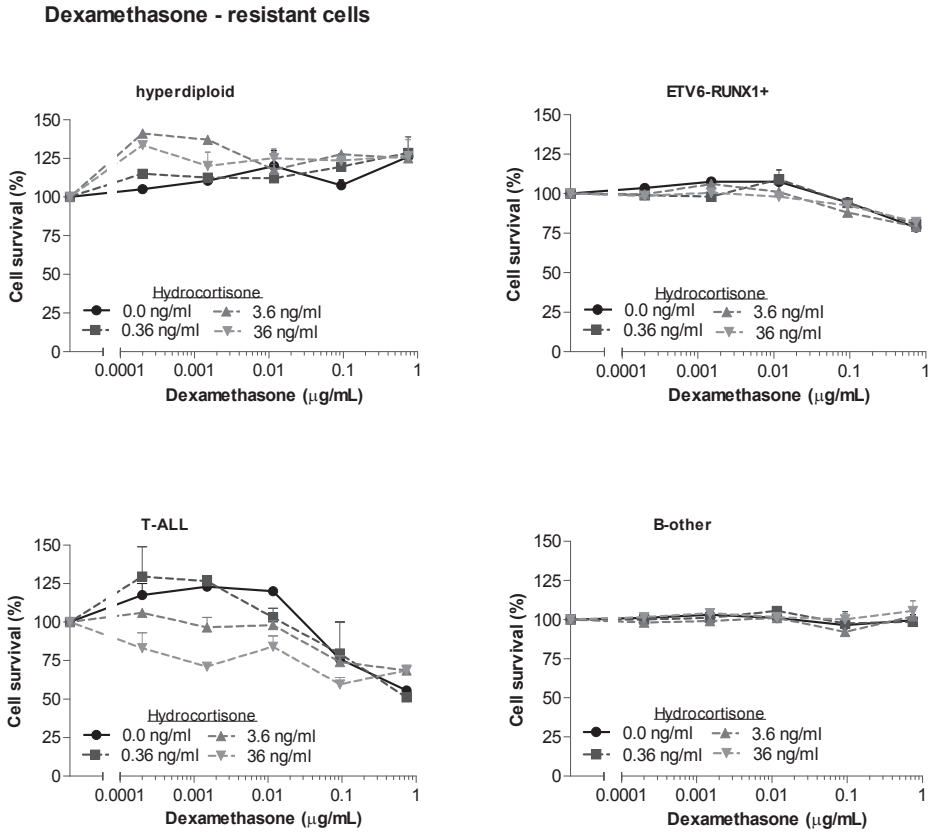

Figure S7. *Hydrocortisone does not induce more resistance to leukemic patients' cells which are intrinsic resistant to dexamethasone in different subtypes of ALL.*

Effect of hydrocortisone (0 ng/ml, 0.36 ng/ml, 3.6 ng/ml, 36 ng/ml) on dexamethasone sensitivity of glucocorticoid resistant primary patients' cells (hyperdiploid, ETV6-RUNX1+, T-ALL, B-other). Data are presented as mean plus SEM. Responsiveness of leukemic cells was determined by an MTT-assay. The cell survival of dexamethasone-exposed cells was corrected for cell death induced by hydrocortisone to determine the sensitizing effect of hydrocortisone.

SUPPLEMENTAL METHODS

Methods microarrays

RNA was extracted by means of Trizol isolation (Invitrogen, Bleiswijk, Netherlands) according to the manufacturer's protocol and RNA quality and integrity determined with the 2100 bioanalyzer (Agilent, Amstelveen, Netherlands). The Affymetrix One-Cycle cDNA Synthesis kit (Santa Clara, CA, USA) and the GeneChip IVT Labeling kit (Santa Clara, CA, USA) were used to synthesize cRNA. RNA processing and hybridization to the Affymetrix U133 Plus 2.0 GeneChip oligonucleotide microarray were performed according to the manufacturer's protocol. Gene-expression values were calculated with Affymetrix Microarray Suite version 5.0. Expression signals were scaled to the target intensity of 500 and log transformed. Only arrays with scaling factor <10 and GAPDH cRNA integrity (3'/5') <3 were used for subsequent normalization procedures and analysis of GR and MR expression levels. Normalization of Affymetrix U133 Plus 2.0 data was performed by Robust Multichip Average and variance stabilization and normalization 2 (VSN2) in the R environment (*Huber et al. Bioinformatics 2002*). Probe sets 232431_at for GR and 205259_at for MR were used to determine the expression levels of both receptors in leukemic cells.

Methods RT-qPCR

RNA was extracted using Trizol isolation (Invitrogen), where after cDNA was synthesized according to standardized procedures. *(Ariës, Leukemia 2014)* NR3C1 (GR) and NR3C2 (MR) mRNA levels were quantified by incorporation of SYBR Green (Thermo Scientific) by RT-qPCR (Applied Biosystems 7900HT). Primers for NR3C1 were; 5'-TGC-CAA-GGA-TCT-GGA-GAT-GA -3' (forward) and 5'-TGG-GAG-GTG-GTC-CTG-TTG-T-3' (reverse). Primers for NR3C2 were; 5'-TCC-CTT-CTG-CTA-TTG-TTG-3' (forward) and 5'-TCC-CCa-CAC-ACC-AAA-CAT-ATT-3' (reverse). Primers used for the reference gene *RPS20*, were 5'-AAGGGCTGAGGATTTTTG-3' (forward) and 5'-CGTTGCGGCTTGTTAG-3' (reverse).

Chapter 4

Hydrocortisone as an intervention for dexamethasone-induced side effects in pediatric acute lymphoblastic leukemia patients: Results of a double blind randomized controlled trial

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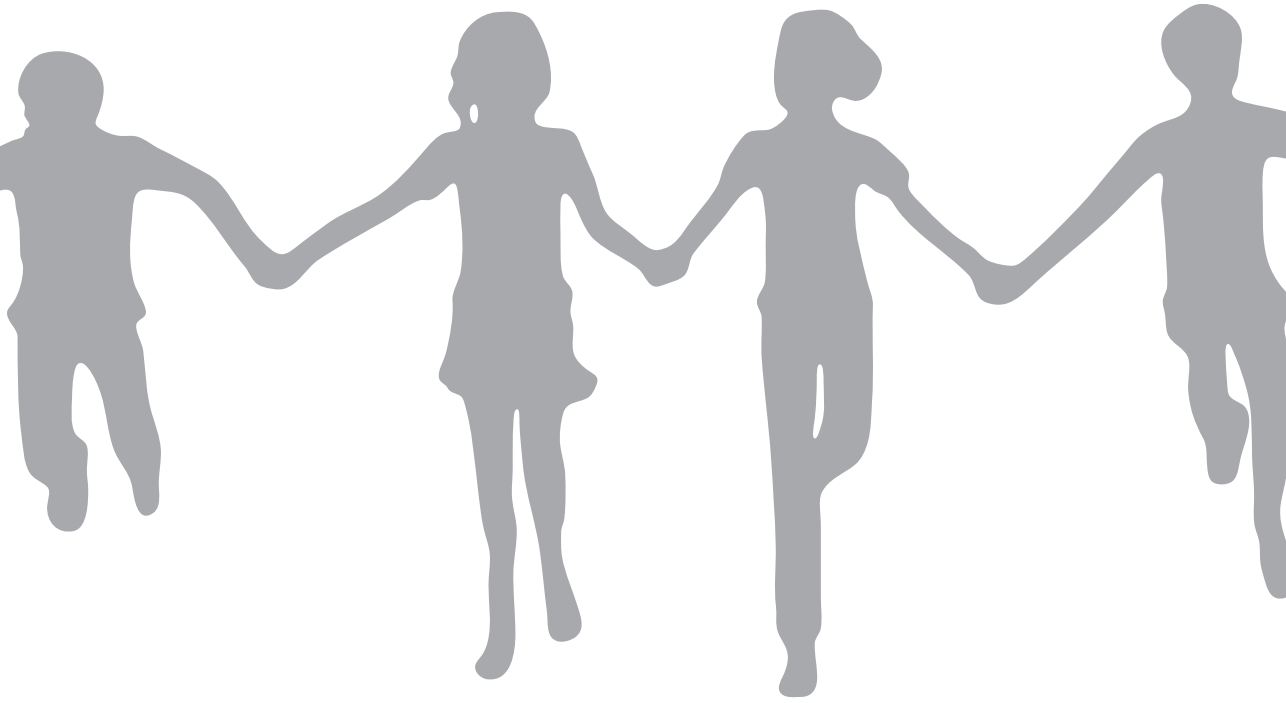

J Clin Oncol. 2016 Jul;34(19):2287-2293.

ABSTRACT

Purpose: Dexamethasone is a key component in treating pediatric acute lymphoblastic leukemia (ALL), but can induce serious side effects. Recent studies have led to the hypothesis that neuropsychological side effects may be due to cortisol depletion of the cerebral mineralocorticoid receptors. We examined whether including a physiological dose of hydrocortisone in dexamethasone treatment can reduce neuropsychological and metabolic side effects in children with ALL.

Patients and Methods: We performed a multicenter double blind, randomized controlled trial with a cross-over design. Fifty out of 116 potentially eligible patients (3-16 years) were enrolled and were treated with two consecutive courses of dexamethasone in accordance with Dutch Childhood Oncology Group ALL protocols. The patients were randomly assigned to receive either hydrocortisone or placebo in a circadian rhythm (10 $mg/m^2/day$) during both dexamethasone courses. The primary outcome measure was the parent-reported Strength and Difficulties Questionnaire-Dut (SDQ), which assesses psychosocial problems. Other endpoint variables included questionnaires, neuropsychological tests, and metabolic parameters.

Results: Of the 48 patients who completed both courses, hydrocortisone had no significant effect on outcome. However, a more detailed analysis revealed that in 16 patients who developed clinically relevant psychosocial side effects, the addition of hydrocortisone substantially reduced their SDQ scores in the following domains: Total Difficulties, Emotional Symptoms, Conduct Problems, and Impact of Difficulties. Moreover, in nine patients who developed clinically relevant sleep-related difficulties, the addition of hydrocortisone reduced Total Sleeping Problems and Disorders of Initiating and Maintaining Sleep. In contrast, hydrocortisone had no effect on metabolic parameters.

Conclusion: Our results suggest that adding a physiological dose of hydrocortisone to dexamethasone treatment can reduce the occurrence of serious neuropsychological side effects and sleep-related difficulties in pediatric ALL patients.

INTRODUCTION

Dexamethasone has high anti-leukemic activity and excellent penetration into the central nervous system; thus, dexamethasone is commonly included in the treatment of pediatric acute lymphoblastic leukemia (ALL).(1-4) Unfortunately, however, dexamethasone treatment can cause robust neuropsychological and metabolic side effects. The reported frequency of patients who develop dexamethasone-related side effects regarding mood, behavior, cognition, and sleep ranges from 5-75%.(5-10) Importantly, patients and their families report that these side effects are the most detrimental with respect to quality of life.(5-11) Because many current ALL treatment protocols call for pediatric ALL patients to receive pulses of dexamethasone for approximately one and a half years, these side effects can have a major impact on the child's daily activities and development.(5, 9, 12) To date, only one intervention study has been performed to investigate glucocorticoid-induced neuropsychological side effects; this study found that chlorpromazine and lorazepam reduced glucocorticoid-related symptoms.(13) However, because these agents can induce other side effects, including drowsiness, orthostatic hypotension, and paradoxical agitation, they should therefore only be prescribed for severe behavioral problems and/or psychosis.

Until recently, the pathophysiology of dexamethasone-related neuropsychological side effects was poorly understood.(7) For example, excessive activation of cerebral glucocorticoid receptors (GRs) by corticosteroid binding has been suggested to underlie the neuropsychological side effects.(7) However, recent data revealed that mineralocorticoid receptors (MRs) in the brain may play an even more important role in regulating mood, behavior, cognition, and sleep.(14, 15) In the human brain, GRs and MRs have similar expression patterns; however, these two receptor types have strikingly different ligand affinities.(16) For example, dexamethasone has a 30-40 fold higher affinity for the GR than cortisol, whereas dexamethasone does not bind to the MR. In contrast, prednisolone binds the GR, but has a low affinity for the MR.(17) Finally, cortisol (i.e. hydrocortisone) can bind both receptor types, but has a higher affinity for the MR.(18) Both dexamethasone and prednisolone suppress the production of cortisol via a negative feedback loop acting on the hypothalamus-pituitary-adrenal axis.(17) However, prednisolone –but not dexamethasone- can bind and activate the MR; thus, patients treated with dexamethasone have fewer cortisol-bound MRs, which may lead to more side effects.(19) Data from animal studies and small case series suggest that the resulting dexamethasone-induced cortisol depletion of MRs in the brain causes or exacerbates the side effects with respect to mood, behavior, and/or cognition.(14, 15, 20, 21)

These key findings led us to hypothesis that dexamethasone-induced cortisol depletion of the MR, may underlie the neuropsychological side effects in pediatric ALL patients.(22) Thus, we examined whether these side effects can be reduced by adding physiological dosages of hydrocortisone to dexamethasone treatment.(22) Importantly, we previously reported that hydrocortisone does not reduce dexamethasone sensitivity of *ex vivo* ALL patients' cells.(22) Therefore, we performed a randomized controlled trial to determine whether including hydrocortisone in the dexamethasone treatment regimen reduces the neuropsychological, metabolic, and physical side effects in children with ALL.

METHODS

Study design and participants

We performed a randomized, placebo-controlled, double blind trial with a crossover design (Figure 1). The primary objective of the study was the reduction of psychosocial problems during dexamethasone treatment. The secondary objective was to study the influence of hydrocortisone addition during dexamethasone treatment on sleep-related difficulties, eating behavior, physical activity, cognitive functions, and metabolic parameters. Patients were recruited at five Dutch pediatric oncology departments. ALL patients (3-16 years) treated according to the Dutch Childhood Oncology Group (DCOG) ALL-10 or ALL-11 medium risk protocols, including dexamethasone pulses during the maintenance phase (after asparaginase and anthracyclines were discontinued), were eligible for inclusion. The following exclusion criteria were applied: a significant language

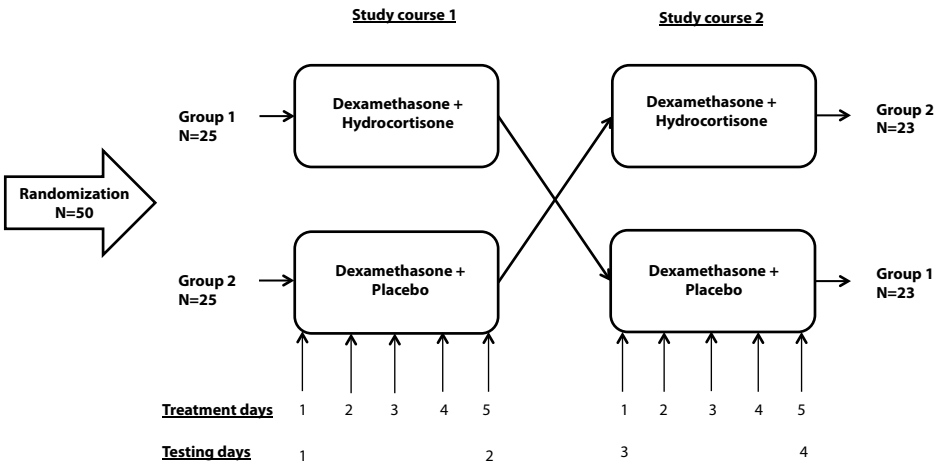

Figure 1. *Study design.*

barrier; evidence of pre-existing intellectual disability; and any condition that could have interfered with the administration and/or absorption of the study medication and/or dexamethasone. Parents and legal guardians of patients provided written informed consent; patients 12-16 years of age also provided their own written informed consent.

The part of maintenance phase in which the intervention was conducted, consists of 19 consecutive treatment cycles lasting 21 days each, in which patients receive five consecutive days of dexamethasone treatment, vincristine once (first day of the cycle), 6-mercaptopurine daily and methotrexate once per week. The study included two 5-day courses of dexamethasone (6mg/m^2/day: 3 doses containing 2 mg/m^2 each) in which each patient also received either placebo or hydrocortisone; in this cross-over study, patients who were randomized to receive hydrocortisone in the first course received placebo in the second course, and vice versa (Figure 1). The median start of the study was in the fourth cycle after stop of asparaginase. The median time between the two 5-day study courses was 3.0 weeks (Inter Quartile Range (IQR): 3.0, 6.0) or one cycle. The daily dose of hydrocortisone was administered orally in three doses (containing 5, 3, and 2 mg/m^2) at the same time as dexamethasone, and was designed to follow the normal circadian rhythm. Placebo was administered in a similar dose and scheme as hydrocortisone. A washout period of ≥16 days was included between the dexamethasone treatment courses.

Procedures

The primary endpoint was the "Total Difficulties" score from the parent-reported Strengths and Difficulties Questionnaire in Dutch (SDQ-Dut). (Table S1) The secondary endpoints were obtained from additional questionnaires, neuropsychological tests (see Table S1 and Supplemental), and metabolic parameters. In each course, mood, behavior, cognition, and sleep were assessed on the morning of treatment day 1 (i.e., before the start of dexamethasone treatment) and the morning of treatment day 5 (i.e., after four full days of dexamethasone treatment).

Questionnaires

The parent-reported SDQ-Dut, which assesses psychosocial difficulties and strengths, has been validated in the Dutch population. The SDQ-Dut(23-26) is a brief questionnaire that assesses the psychosocial functioning of children and adolescents (3-16 years of age) by either parent reporting or self-reporting (patients 11-16 years of age). The questionnaire contains 25 items in the following five subscales (see Supplemental for score ranges): Emotional Symptoms, Conduct Problems, Hyperactivity and Inattention, Peer Relationship Problems and Prosocial Behavior. The Total Difficulties score, defined as the sum of the first four subscale scores (i.e., excluding Prosocial Behavior) was calculated.

The impact of these difficulties on the child's life was measured using the Impact of stress score. A higher SDQ Total Difficulties score reflects more problems. Ideally, both parents and all patients ≥11 years of age completed the SDQ-Dut on all four testing days. On each testing day, the participants were instructed to provide information regarding psychosocial problems experienced in the previous four days. The SDQ scores obtained from the primary parent (defined as the parent who was present in the outpatient clinic at all four testing days) were used for all analyses; in the majority of cases, the primary parent was the patient's mother.

The Sleep Disturbance Scale for Children (SDSC)(27) was used to assess sleep quality and sleep disturbances in the patients. The SDSC has a combined score that covers the six most common sleep disorders experienced during childhood; these disorders are Disorders of Initiating and Maintaining Sleep (DIMS), Sleep Breathing Disorders (SBD), Disorders of Arousal (DA), Sleep-Wake Transition Disorders (SWTD), Disorders of Excessive Somnolence (DES), and Sleep Hyperhidrosis (SHY). A higher score reflects the presence of more problems.

The Dutch Eating Behavior Questionnaire for children (DEBQ-C)(28) has three subscales: Restrained Eating, Emotional Eating, and External Eating; a higher score on each subscale reflects the presence of more problems.

The daily physical activity was measured using the Baecke Physical Activity Questionnaire(29)(BPAQ), which consists of 16 questions organized in three sections: school activity, sports activity, and leisure activity. With the BPAQ, a higher score on each scale reflects higher activity.

Neuropsychological assessment

Neuropsychological tests designed for children and young adults were used to assess skills in four domains (memory, attention, visual-spatial functions, and processing speed; Table S1). The neuropsychological tests were performed by the same investigator (author LW) on all four testing days.

Physical parameters, anthropometric measurements and laboratory tests

Parents and children were instructed to maintain a diary of the child's dietary activity during the first four treatment days in each study courses. (Supplemental) Height (meters), weight (kg), waist-hip circumference (cm) and blood pressure (mmHg) were measured on all four testing days.

Physical activity was measured throughout both courses using a Philips DirectLife activity monitor.(30) Fasting blood samples (whole blood) were taken between 8am

and 10am and used to analyze lipid profiles (triglycerides, cholesterol, HDL, and LDL), glucose and insulin levels.

Adverse events

Adverse events (i.e., any adverse change in condition between the first dose and 16 days after the last dose) were assessed in accordance with the US National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE), version 4.0.(31)

Statistical analysis

The data were analyzed for carry-over effects and for period (i.e., order of treatment) effects using the paired Student's *t*-test (each patient served as his/her own control). (32) In each treatment course, a delta-score (i.e., the difference between two scores) was calculated by subtracting the score on treatment day 1 from the score on treatment day 5. The treatment effect was assessed by comparing the delta-placebo score with the delta-hydrocortisone score using the paired Student's *t*-test (normally distributed values) or the Wilcoxon-signed-rank test. Adjusted *P*-values (Benjamin-Hochberg procedure) are reported in Table S8. A nested subset analysis was used to evaluate the effect of hydrocortisone in children who experienced clinically relevant dexamethasone-related side effects. Clinically relevant psychosocial side effects were defined as a change in parent-reported SDQ Total Difficulties score of ≥5 during the patient's respective placebo course; this difference represents approximately one standard deviation in the general population(23). Clinically relevant sleeping problems were defined as a change in the Total SDSC score ≥7 (one standard deviation) during the patient's respective placebo course.(27, 33) In the subset analysis, we examined the effect size rather than the *P*-value because of the potential influence of regression to the mean in the subgroup selection.

RESULTS

Enrollment

During the recruitment period, 50 out of 116 potentially eligible patients (49.5%) enrolled at the five pediatric oncology departments from July 2012 through February 2015. The most frequently cited reasons for not participating in the study were the high burden of two extra visits (37 patients) and an absence of *a priori* dexamethasone-related side effects (8 patients). (Figure 2) After the patients were randomly assigned to the treatment groups, two patients left the trial after the first course due to dexamethasone-related osteonecrosis; these patients were excluded from the efficacy analyses.

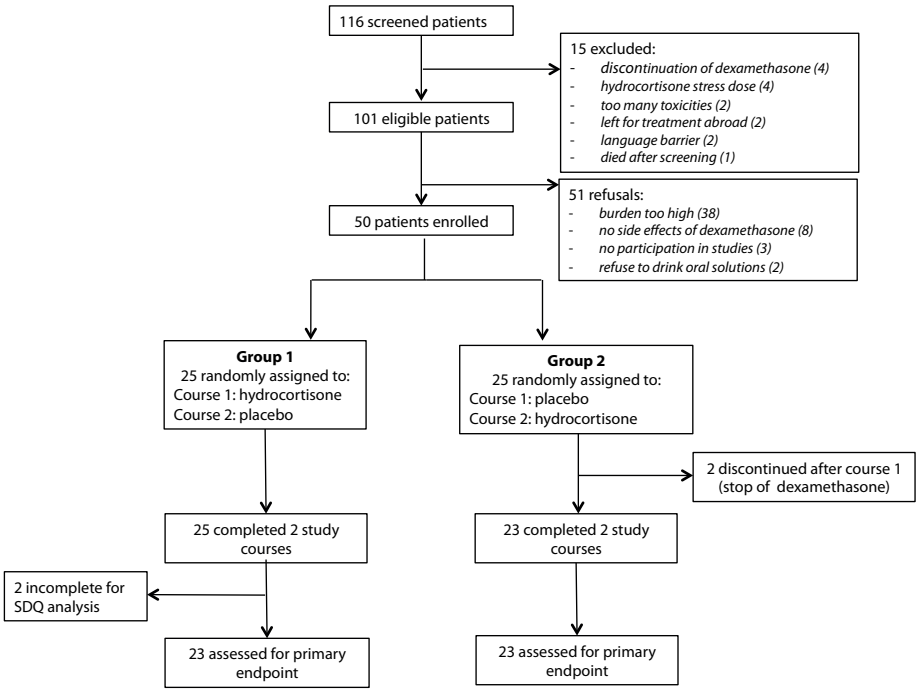

Figure 2. *Patient eligibility and randomization.*

The treatment groups were similar with respect to age, type of leukemia, treatment protocol, and central nervous system status at diagnosis. (Table S2) Two patients did not complete the parent-reported SDQs at all four time points and were therefore excluded from the efficacy analysis. Four patients developed serious adverse events after the first study course (2 hydrocortisone courses; 2 placebo courses); three of these patients developed febrile neutropenia (grade 2-3), and one patient developed osteomyelitis (grade 3). These serious adverse events were not considered related to study medication, and all four patients remained in the study. The adverse events were similar between the hydrocortisone and placebo courses, indicating that no hydrocortisone-specific adverse events were observed. (Table S3) No carry-over effect (P=0.34; independent samples Student's t-test) or period effect (P=0.76; Mann-Whitney test) was observed based on the primary outcome.

Psychosocial problems

The SDQs results obtained from 46 primary parents (41 mothers and 5 fathers) were analyzed in order to evaluate the psychosocial problems of the children. Four days of dexamethasone treatment significantly increased the patients' problems as reported by all SDQ scales and subscales. In 30 of the 46 patients (65%), dexamethasone induced an increase in psychosocial problems (defined as a change in the SDQ Total difficulties

score of ≥1) during the placebo course. One third of the population did not have any increase in SDQ Total difficulties on dexamethasone. The median SDQ "Total difficulties" score of the entire study group on treatment day 5 in the placebo course was 9.5, which is within the normal range.

In the entire group, addition of hydrocortisone did not affect the "Total difficulties" score (mean difference was -0.8±5.5; *P*=0.33), "Emotional symptoms" (mean difference was -0.6± 2.3; *P*=0.08), "Conduct problems" (mean difference was 0.0±1.5; *P*=1.00) or the other SDQ subscales compared to the placebo course. (Figure 3)

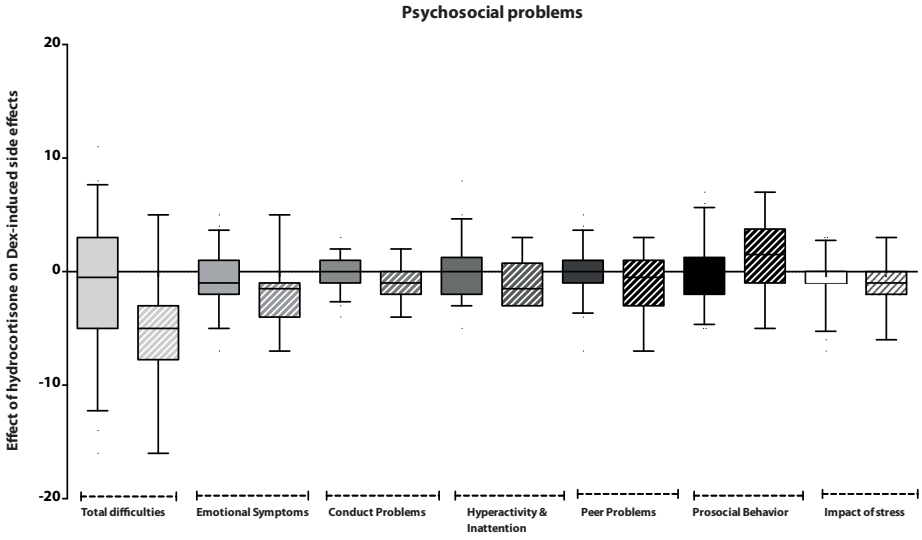

Figure 3. *The effect of hydrocortisone addition in the total group (N=46)(left bars with no pattern) and in the patients (N=16)(right bars with pattern) with clinically relevant dexamethasone-induced psychosocial side effects on SDQ subscales. Effect was measured by: delta hydrocortisone minus delta placebo. Delta = score treatment day 5 minus score treatment day 1. Box-Whisker plots with median and 5-95 percentiles are depicted for each SDQ scale. A negative score reflects a decrease in side effects by hydrocortisone, with exception of the Prosocial Behavior score (positive score reflects less side effects).*

However, when we examined the effect of hydrocortisone on the subset of 16 patients who had clinically relevant dexamethasone-related side effects (i.e., an increase in their SDQ "Total difficulties" score≥5), we found that hydrocortisone had a clinically significant treatment effect. In these 16 children hydrocortisone had a clear effect on the "Total difficulties" delta-score compared to placebo (the median difference was -5.0; Inter Quartile Range (IQR): -7.8 to -3.0) (Figure 4). In five of these 16 patients (31%) the "Total difficulties" score decreased from a high score in the placebo course to a score in the normal range with the addition of hydrocortisone. We also observed a significant effect of hydrocortisone versus placebo on "Emotional symptoms" (median difference

was -1.5; IQR: -4.0 to -1.0), "Conduct problems" (median difference was -1.0; IQR: -2.0 to 0.0), and "Impact of stress" (median difference was -1.0; IQR: -2.0 to 0.0) (Figure 4). (See Table S4-5 for real SDQ scores and for point estimates (95% confidence intervals) of the median differences)

With respect to their baseline characteristics, the patient group with clinically relevant dexamethasone-induced side effects did not differ significantly from the group of patients without clinically relevant dexamethasone-induced psychosocial side effects. The week of maintenance phase in which the patients participated did not influence the side effects. (P=0.47) The child-reported SDQ scores (N=10) did not differ significantly from their respective parent-reported scores (*P*=0.44).

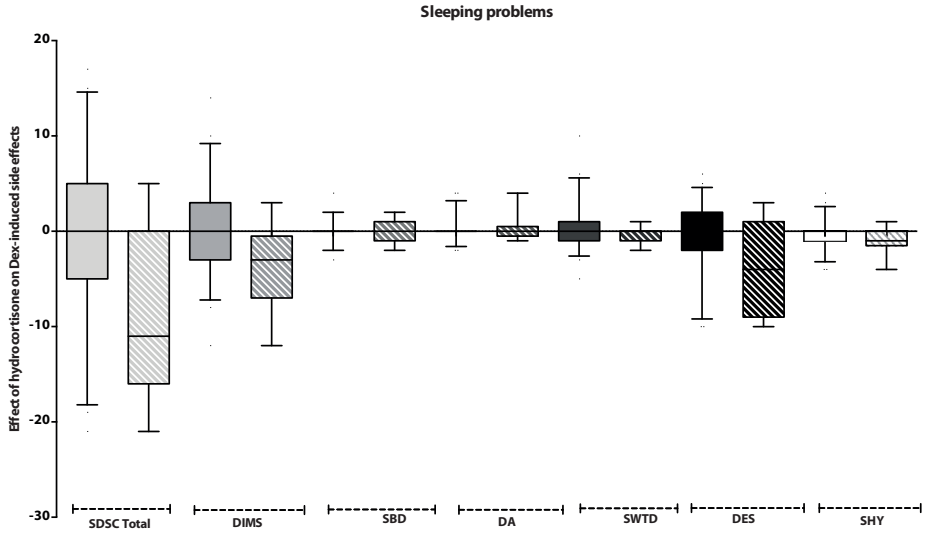

Figure 4. *The effect of hydrocortisone addition in the total group (N=47) (left bars with no pattern) and in the patients (N=9) (right bars with pattern) that suffer from clinically relevant dexamethasone-related sleeping problems on the SDSC subscales. Effect was measured by: delta hydrocortisone minus delta placebo. Delta= score treatment day 5 minus score treatment day 1. Box-Whisker plots with median and 5-95 percentiles are depicted for each SDSC scale. A negative score reflects a decrease in side effects by hydrocortisone.*

Sleep

The parents of 47 children completed the SDSC questionnaire on all four testing days. Dexamethasone treatment alone (i.e., the placebo course) significantly increased the DA (*P*=0.04), SWTD (*P*=0.01) and DES (*P*=0.01) scores. In the entire patient group, hydrocortisone had no significant effect on the SDSC scores (SDSC Total score: *P*=0.84, DIMS: *P*=0.74, DES: *P*=0.29, SWTD: *P*=0.29). (Figure 5) However, when the 9 children (19%) who had clinically relevant dexamethasone-induced sleeping problems (defined as a change

in SDSC Total score≥7 during the placebo course) were analyzed separately, hydrocortisone reduced both the SDSC Total scores (median difference was -11.0; IQR: -16.0 to 0.0) and DIMS scores (median difference was -3.0; IQR: -7.0 to -0.5) (Figure 6). The majority of patients with clinically relevant sleeping problems also experienced clinically relevant psychosocial side effects during dexamethasone (N=7, 78%).

Neuropsychological functioning

Neuropsychological tests revealed that dexamethasone treatment alone had no effect on attention (auditory attention, response set, and inhibition), visual-spatial functions (design copying), memory (narrative memory and memory for designs) or processing speed. However, the addition of hydrocortisone significantly improved long-term visual memory (MDDT; *P*=0.01,*N*=47) (Table S4); hydrocortisone had no effect on the other neuropsychological tests of attention, visual-spatial function (NEPSY), or processing speed (Wechsler). (Table S6) The neuropsychological performance of the children with clinically relevant dexamethasone-induced psychosocial side effects was similar with the neuropsychological performance of the entire group.

Metabolism

Physical activity data (measured using the BPAQ) were available for 36 patients, and activity monitor data were available for 41 patients. Physical activity was neither affected by dexamethasone, nor by hydrocortisone addition.

Dietary intake and data regarding eating behavior (measured using the DEBQ-C) were available for 44 and 17 patients, respectively (Table S1). Hydrocortisone had no significant effect on energy intake (*P*=0.88). Similarly, including hydrocortisone had no significant effect on weight, height, waist/hip ratio, blood pressure, or any laboratory values (Table S7).

DISCUSSION

Here, we report the results of the first randomized controlled clinical trial to investigate whether a potentially safe intervention (i.e., physiological doses of hydrocortisone) can be used to reduce dexamethasone-induced neuropsychological side effects in pediatric ALL patients. Both patients and their parents consider neuropsychological side effects to be the most detrimental consequences of ALL treatment with respect to reducing quality of life.[2] Our results show that although hydrocortisone had no significant beneficial effect in the entire patient group, hydrocortisone significantly decreased dexamethasone-related behavioral difficulties, emotional disorders, and sleep problems specifically in patients who experienced the most severe neuropsychological side effects. This finding is particularly relevant, as psychosocial problems can be present in up to two-thirds of

children with ALL, and half of the problems can be categorized as "clinically relevant". Thus, our results indicate that these emotional and behavioral problems can be reduced in these children, thereby markedly improving quality of life.(34) Moreover, sleeping problems –half of which were categorized as "clinically relevant"- have been reported in 43% of patients, and reducing these problems may also improve quality of life.(35)

The flipside of these findings is, that adding hydrocortisone does not benefit all children with ALL. One third of the population did not have any neuropsychological side effects on dexamethasone. This patient variability on side effects may be explained by genetics(36), glucocorticoid sensitivity[39] or dexamethasone clearance (higher drug levels)(37). The occurrence of neuropsychological side effects was independent of age, in contrast to Mrakotsky *et al.* who reported more neurobehavioral problems in younger children. (38) It needs to be mentioned that subgroups are small and that regression to the mean could have influenced our subgroup selection and that our results should be confirmed preferably in a validation study in selected patients with symptoms only. However, the substantial effect size of the intervention indicates a benefit of hydrocortisone in patients with psychosocial problems and sleeping problems.

Hydrocortisone addition did improve one specific memory score; due to the absence of acute dexamethasone-induced impairment on cognitive function, the clinical relevance of this finding is limited. The absence of dexamethasone-induced short-term cognitive impairment is in accordance with the study of Wingenfeld *et al(39)* who did not find an effect of high-dose dexamethasone on working memory in healthy volunteers.

Interestingly, hydrocortisone also had no effect on the metabolic side effects of dexamethasone. This lack of efficacy may be caused by a different pathophysiology of metabolic side effects. This notion is supported by the absence of a significant difference in body weight change during induction therapy -an important metabolic side effect of high dose glucocorticoids (e.g., prednisolone)- between children with neuropsychological side effects and children without neuropsychological side effects. It is also conceivable that metabolic side effects are not caused by cortisol depletion of the cerebral MRs.

In conclusion, the current study suggests that including a physiological dose of hydrocortisone decreases clinically relevant dexamethasone-induced psychosocial problems and sleeping problems in pediatric ALL patients. For a validation study it is important to identify these patients that benefit from hydrocortisone using the SDQ-Dut and the SDSC. Physiological doses of hydrocortisone are relatively inexpensive, provide a naturally occurring hormone, and have no apparent negative effects. This novel yet simple intervention has the potential to significantly reduce neuropsychological side effects in patients receiving high-dose dexamethasone treatment.

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SUPPLEMENTAL TABLES.

Neuropsychological assessment

Table S1. *Overview of questionnaires and neuropsychological tests and the corresponding subjects who completed or performed the test.*

Questionnaire / Test	Completed by:
SDQ-Dut parent	Parent (child 3-16 yrs)
SDQ-Dut child	Children 11-16 yrs
SDSC	Parent (child 3-16 yrs)
DEBQ-C	Children 7-12 yrs
BPAQ	Parent (child 3-11 yrs) Children 12-16 yrs
NEPSY AA	Children 5-16 yrs
NEPSY RS	Children 7-16 yrs
NEPSY INE	Children 5-16 yrs
NEPSY DC	Children 3-16 yrs
NEPSY NM	Children 3-16 yrs
NEPSY MDT	Children 3-16 yrs
NEPSY MDT delayed	Children 5-16 yrs
Wechsler WPPSI III	Children 3-6 yrs
Wechsler WISC III	Children 7-16 yrs

Baseline Characteristics

Table S2. *Baseline characteristics of the enrolled patients (N=50).*

	Group 1: Hydrocortisone-Placebo N=25	Group 2: Placebo-Hydrocortisone N=25	P-value
Age at assessment (y)	9.0 (5.5-12.0)	5.0 (3.5-7.0)	0.09
Sex			
female	13 (52)	14 (56)	0.78
male	12 (48)	11 (44)	
Week of maintenance phase	34.0 (31.0-42.0)	40.0 (34.0-49.5)	0.15
ALL subtype			1.00
B-ALL	23 (92)	23 (92)	
T-ALL	2 (8)	2 (8)	
CNS status at diagnosis			0.57
CNS-1	12 (48)	10 (40)	
CNS-2	13 (52)	15 (60)	
CNS-3	0 (0)	0 (0)	

Data are shown as median (inter quartile range) or as number (%). CNS = central nervous system. CNS 1 = ≤ 5 WBC/µl and central nervous system fluid (CSF) with ≤ 1 leukemic cells, CNS 2 = ≤ 5 WBC/µl and CSF with ≥ 2 identifiable leukemic cells, CNS 3 = > 5 WBC/µl and CSF with identifiable leukemic cells.

Table S3. *Adverse events according to the NCI CTCAE version 4.0[33] after 4 full days of hydrocortisone or placebo administration. N=48. Data are depicted as number (%).*

	Hydrocortisone day 5	***Placebo day 5***	***P-value***
Cushingoid grade 1	10 (21)	12 (25)	0.16
Abdominal pain grade 1	7 (15)	5 (10)	0.42
Agitation grade 1	5 (10)	2 (4)	0.53
grade 2	2 (4)	1 (2)	
grade 3	0 (0)	1 (2)	
Nausea grade 1	1 (2)	3 (6)	0.32
Constipation grade 1	3 (6)	3 (6)	0.49
grade 2	2 (4)	1 (2)	
Diarrhea grade 1	1 (2)	0 (0)	0.32
Skin rash	0 (0)	1 (2)	0.32
Sensible neuropathy	0 (0)	1 (2)	0.32
Pain grade 1	5 (10)	5 (10)	1.00
Tiredness grade 1	22 (46)	16 (33)	0.74
grade 2	8 (17)	10 (21)	
grade 3	1 (2)	1 (2)	

Grade 1 = mild; intervention not indicated. Grade 2 = moderate; local or non-invasive intervention indicated. Grade 3 = severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated.
The difference between the hydrocortisone and placebo course was tested with use of a Paired T-test.

Table S4. *Overview of parent-reported SDQ scores (Median (Inter Quartile Range)) of patients with delta-SDQ overall stress≥5 in the placebo course (N=16). More dexamethasone-induced problems is reflected by a higher delta score, except for 'Prosocial behavior' which becomes lower.*

	Hydrocortisone day1	***Hydrocortisone day5***	***Placebo day1***	***Placebo day5***
Total difficulties	7.5 (4.5, 11.8)	12.5 (9.3, 16.8)	7.0 (4.3,11.0)	17.0 (12.3, 24.3)
Emotional symptoms	2.0 (1.3, 5.0)	5.5 (2.0, 6.8)	2.5 (1.0, 4.0)	6.0 (4.0, 8.8)
Conduct problems	1.0 (0.0, 3.8)	3.0 (1.0, 4.0)	1.0 (0.0, 3.0)	3.5 (2.3, 4.0)
Hyperactivity and Inattention	3.0 (1.0, 4.8)	3.5 (3.0, 5.0)	3.0 (0.3, 4.8)	5.0 (3.0, 6.8)
Peer relationship Problems	1.0 (0.3, 1.8)	2.0 (1.0, 2.8)	1.0 (0.0, 1.8)	3.0 (1.0, 4.0)
Prosocial behavior	7.5 (6.0, 9.0)	4.5 (3.0, 7.0)	7.5 (6.3, 9.8)	4.0 (3.0, 6.0)
Impact of stress	0.0 (0.0, 1.8)	2.5 (1.0, 4.8)	0.0 (0.0, 3.5)	4.0 (2.3, 6.8)

Table S5. *The point estimates of the median differences and their Binomial-based 95% confidence intervals of parent-reported SDQ scores of patients with delta-SDQ overall stress≥5 in the placebo course (N=16) and parent-reported SDSC-scores of patients with delta-SDSC total score ≥7 during the placebo course (N=9).*

	Point estimate median difference	***95% confidence interval***
Parent-reported SDQ-scores		
Total difficulties	-5.0	-8.0, -3.0
Emotional symptoms	-1.5	-4.0, -1.0
Conduct problems	-1.0	-2.0, 0.0
Hyperactivity and Inattention	-1.5	-3.0, 1.0
Peer relationship Problems	-0.5	-3.0, 1.0
Prosocial behavior	1.5	-1.0, 4.0
Impact of stress	-1.0	-2.0, 0.0
Parent-reported SDSC-scores		
SDSC Total	-11	-17, 1
DIMS	-3	-8, 2
SBD	0	-2, 2
DA	0	-1, 1
SWTD	0	-1, 0
DES	-4	-10, 1
SHY	-1	-2, 0

DIMS = Disorders of Initiating and Maintaining Sleep, SBD = Sleep Breathing Disorders, DA = Disorders of Arousal, SWTD = Sleep-Wake Transition Disorders, DES = Disorders of Excessive Somnolence, SHY = Sleep Hyperhidrosis.

Neurocognitive function

Table S6. *Effect of hydrocortisone on neurocognitive test scores (Median (IQR)) of the total study group.*

	Effect of hydrocortisone	***P-value***
AA (N=32)	0.0 (-2.0, 0.8)	0.25
RS (N=21)	1.0 (-0.5, 2.0)	0.16
INE (N=30)	-1.0 (-2.0, 4.0)	0.79
DC general (N=48)	0.0 (-1.0, 1.8)	0.71
DCPT (N=48)	0.0 (-8.8, 5.8)	0.79
NMFC (N=41)	1.0 (-4.0, 5.0)	0.63
NMRG (N=47)	0.0 (-1.0, 1.0)	0.90
MDT (N=47)	0.0 (-3.0, 6.0)	0.52
MDDT (N=30)	0.0 (0.0, 3.0)	0.01
PSI (N=38)	0.0 (-6.8, 8.3)	0.92

AA= auditory attention total correct, RS= response set total correct, INE= inhibition total errors, DC general= design copying total score, DCPT= design copying process total score, NMFC= narrative memory free and cued recall total score, NMRG= narrative memory recognition total score, MDT= memory for designs total score, MDDT= memory for designs delayed total score, PSI= Processing Speed Index.

Effect of hydrocortisone was measured by: delta hydrocortisone minus delta placebo. Delta score= score treatment day 5 minus score treatment day 1. N varies due to differences in validation for age. The treatment effect

of hydrocortisone was tested by comparing delta hydrocortisone with delta placebo with a Wilcoxon Signed Rank test.

Laboratory parameters

Table S7. *Effect of hydrocortisone on HDL, LDL, total cholesterol, insulin, glucose and triglycerides levels (Median (IQR)). Effect of hydrocortisone was measured by: delta hydrocortisone minus delta placebo. Delta score = score treatment day 5 minus score treatment day 1. The treatment effect of hydrocortisone was tested by comparing delta hydrocortisone with delta placebo with a Wilcoxon Signed Rank test.*

	Effect of hydrocortisone	***P-value***
HDL	0.03 (-0.25, 0.18)	0.93
LDL	-0.05 (-0.32, 0.34)	0.55
Total cholesterol	-0.20 (-0.35, 0.30)	0.43
Insulin	-13.10 (-263.65, 94.25)	0.53
Glucose	-0.10 (-0.40, 0.78)	0.70
Triglycerides	-0.07 (-0.38, 0.31)	0.27

Table S8. *Benjamin-Hochberg adjusted P-values for multiple testing.*

Tested hypothesis	***P-value***	***Adjusted P-value***
SDQ: hydrocortisone versus placebo		
Total difficulties	0.33	0.58
Emotional symptoms	0.08	0.22
Conduct problems	1.00	1.00
Influence week maintenance on SDQ Total difficulties	0.47	0.66
SDQ Total difficulties child versus to parent-report	0.44	0.66
SDSC: placebo course day 1 versus day 5		
DA	0.04	0.14
SWTD	0.01	0.047
DES	0.01	0.047
SDSC: delta hydrocortisone versus placebo		
Total score	0.84	0.95
DIMS	0.74	0.94
DES	0.29	0.58
SWTD	0.29	0.58
NEPSY visual memory: delta hydrocortisone versus placebo	0.01	0.047
Energy intake: hydrocortisone versus placebo	0.88	0.95

DIMS = Disorders of Initiating and Maintaining Sleep, DA = Disorders of Arousal, SWTD = Sleep-Wake Transition Disorders, DES = Disorders of Excessive Somnolence.

SUPPLEMENTAL METHODS.

Study Randomization

Patients were recruited at five Dutch pediatric oncology departments: Erasmus Medical Center, Academic Medical Center Amsterdam, VU Medical Center, University Medical Center Utrecht and University Medical Center Groningen.

Subjects were registered via a web-based service called TenALEA, which generated subject numbers. The order in which the patients received placebo and hydrocortisone (Figure 1) was determined randomly and provided by a statistician. The personnel, participants, and clinicians were blinded with respect to the treatment allocation, with the exception of the pharmacy personnel at the participating centers, who had access to the randomization list. Blinding was maintained until the last patient's final visit and after final validation of the online OpenClinica Community edition database.

Power analysis

A power analysis was performed on the primary outcome parameter (the Total difficulties score of the parent-reported Strengths and Difficulties Questionnaire in Dutch). The inclusion of ≥40 children was determined to be sufficient to achieve >80% probability of detecting a significant effect ($\alpha = 0,05$) of the intervention based on a change of 6 points (±1 standard deviation) in the Total Difficulties score. Based on the probability of patients dropping out of the study (20%), we determined that 50 patients were required for this study.

Ethics committee

The study protocol was approved by the local ethics committee at each participating center (MEC-2012-155/ EudraCT 2011-003815-46). In accordance with national regulations, parents and legal guardians of patients provided written informed consent; patients 12-16 years of age also provided their own written informed consent. The study was performed in accordance with the Declaration of Helsinki and followed the principles of good clinical practice. *(WHO guidelines Good Clinical Practice for trials with pharmaceutical products. 1995)* The study was registered in the Dutch Trial Registry (NTR3280).

Strengths and Difficulties Questionnaire (SDQ)

The questionnaire contains 25 items in the following five subscales: Emotional Symptoms (normal: 0-3, mildly elevated: 4, high: 5-10); Conduct Problems (normal: 0-2, mildly elevated: 3, high: 4-10), Hyperactivity and Inattention (normal: 0-2, mildly elevated: 3, high: 4-10), Peer Relationship Problems (normal: 0-5, mildly elevated: 6, high: 7-10) and Prosocial Behavior (normal: 6-10, mildly elevated: 5, high: 0-4). A Total Difficulties score

(normal range: 0-10, mildly elevated: 11-13, high: 14-40) was calculated by summing the first four subscale scores (i.e., excluding Prosocial Behavior).

Sleeping Disturbance Scale for Children (SDSC)

The parent-reported SDSC has been validated for use in children 6-16 years of age; in addition, a pilot study performed in Italy found that the SDSC can also be used for children 3-6 years of age. *(Romeo DM, Bruni O, Brogna C, et al: Application of the sleep disturbance scale for children (SDSC) in preschool age. Eur J Paediatr Neurol 17:374-382, 2013)* We therefore used the SDSC for our entire study population. The total SDSC score is the sum of the 26 included items (on a Likert scale) with a range of 26 -130.

Dutch Eating Behavior Questionnaire (DEBQ)

The DEBQ-C has been validated for use in children 7-12 years of age and was therefore completed by patients 7-12 years of age.

Baecke Physical Activity Questionnaire (BPAQ)

The questions in each section are scored on a 5-point Likert scale. Although the BPAQ has been validated for use only in children 12 years of age and older *(Vogels N, Westerterp-Plantenga MS: Categorical strategies based on subject characteristics of dietary restraint and physical activity, for weight maintenance. Int J Obes (Lond) 29:849-857, 2005*) it has also been used before as a parent-reported questionnaire for children of 3-9 years of age. (*Kaspiris A, Zaphiropoulou C, Vasiliadis E: Range of variation of genu valgum and association with anthropometric characteristics and physical activity: comparison between children aged 3-9 years. J Pediatr Orthop B 22:296-305, 2013*) Therefore, in our study the BPAQ was completed by the parents of children 3-11 years of age and by children 12-16 years of age.

Neuropsychological assessment

Memory (Narrative memory (NMFC and NMRG) and Memory for Designs (MDT and MDDT)), attention (Inhibition (INE), Auditory Attention (AA), and Response Set (RS)), and visual-spatial functions (Design Copying (DC general and DCPT)) were assessed using NEPSY. Processing speed was assessed using the Processing Speed Index (PSI) of the age-appropriated Dutch version of the Wechsler scales (WPPSI-III for children 3-6 years of age and WISC-III for children 7-16 years of age).

Physical parameters, anthropometric measurements and laboratory tests

Parents and children were instructed to maintain a diary of the child's dietary activity during the first four treatment days in each study courses. Food intake was converted to energy intake (in kcals) using a database of the chemical composition of foods (NEVO,

Netherlands Food Composition chart, 2011). Height (in meters), weight (in kg), waist-hip circumference (in cm) and blood pressure (in mmHg) were measured on all four testing days. Weight at diagnosis and at day 30 of induction therapy were retrospectively collected from patient records. Blood pressure was measured in the right arm using a Dinamap Procare with the subject in the sitting position after one hour's of rest.

Physical activity was measured throughout both courses using a Philips DirectLife activity monitor. Fasting blood samples (whole blood) were taken between 8 am and 10 am and used to analyse lipid profiles (triglycerides, cholesterol, HDL, and LDL), glucose and insulin levels. The fasting blood samples were centrifuged and kept frozen at −80°C until analysis as a batch at the end of the study. Serum samples were measured in the same laboratory using an enzymatic *in vitro* assay (Roche Diagnostics, Mannheim, Germany).

Chapter 5

Acute activation of metabolic syndrome components in pediatric acute lymphoblastic leukemia patients treated with dexamethasone

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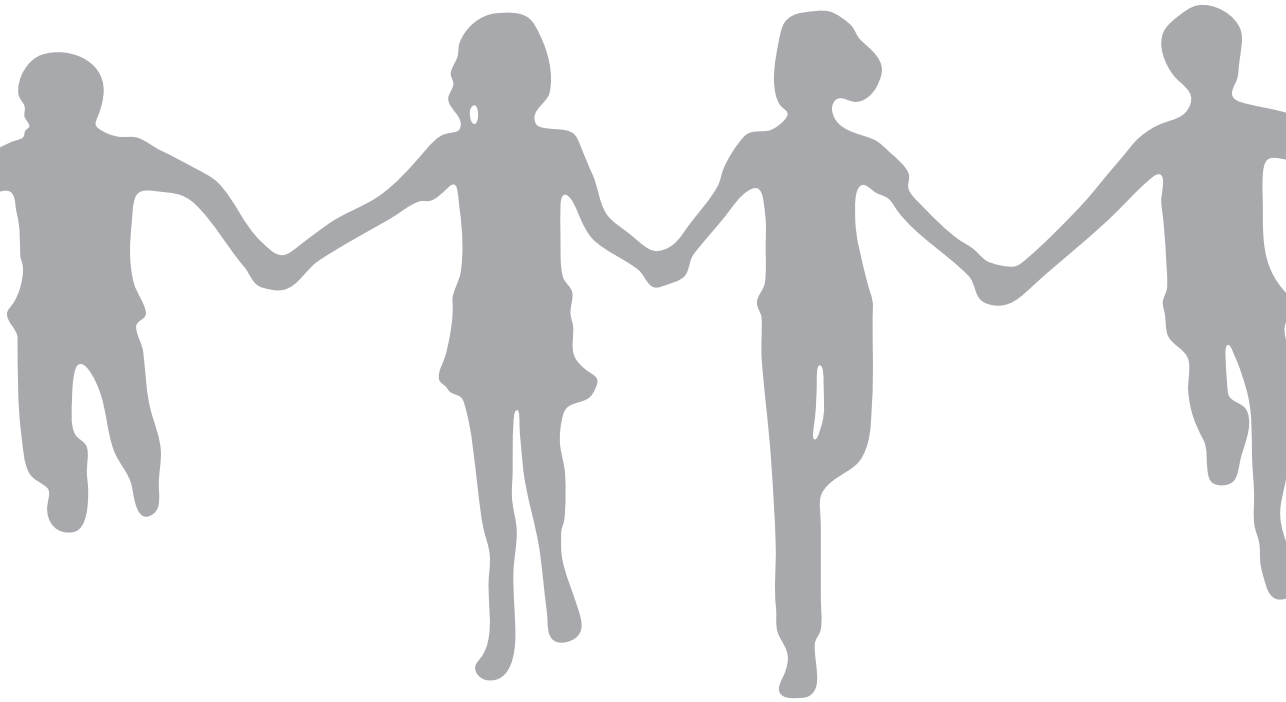

PLoS ONE. 2016 Jun;11(6): e0158225.

ABSTRACT

Dexamethasone, a highly effective drug in the treatment of pediatric acute lymphoblastic leukemia (ALL), can induce serious metabolic side effects. Since data are limited to small studies, we prospectively studied the direct effects of dexamethasone administration on all components of the metabolic syndrome (MetS) in ALL patients, and investigated whether these side effects were dependent on dexamethasone levels.

Fifty ALL patients (3-16 years of age) were included, and were studied during one 5-days dexamethasone course in the maintenance phase of the Dutch Childhood Oncology Group ALL-10 and ALL-11 protocol. Fasting insulin, glucose, total cholesterol, HDL, LDL, triglycerides levels, and anthropometric parameters were measured at baseline before start of dexamethasone (T1), and at day 5 (T2). Dexamethasone trough levels were measured at T2.

Dexamethasone administration significantly increased median fasting serum levels of HDL (1.42 versus 1.55 mmol/L, *P*=0.00), LDL (2.55 versus 2.76 mmol/L, *P*=0.00), total cholesterol (4.20 versus 4.60 mmol/L, *P*=0.00), triglycerides (0.86 versus 1.09 mmol/L, *P*=0.04), glucose (4.4 versus 4.7 mmol/L, *P*=0.00) and insulin (25.2 versus 216.5 pmol/L, *P*=0.00). Insulin resistance (HOMA-IR>3.4) increased from 8% to 85% *(P*=0.00). Dexamethasone also significantly increased diastolic and systolic blood pressure SDS. Dexamethasone trough levels (*N*=24) were positively correlated with high glucose levels at T2, but not with other parameters.

We conclude that dexamethasone induces metabolic toxicity on three components of the MetS, already within four days of treatment. These findings, together with the weight gain during dexamethasone treatment, may contribute to the higher prevalence of MetS and cardiovascular risk childhood leukemia survivors.

INTRODUCTION

Glucocorticoids are an important component of effective pediatric acute lymphoblastic leukemia (ALL) treatment.[1] Unfortunately, high dose dexamethasone used in ALL treatment is notorious for serious side effects.[2, 3] Acute metabolic side effects are more apparent in dexamethasone-based than in prednisone-based schedules.[4, 5]

Metabolic side effects, such as weight gain, altered fat distribution, hypertension, hyperglycemia, dyslipidemia, and altered insulin resistance have been described in 10-45% of children treated with glucocorticoids.[2, 6-8] These effects are amplified by increased energy intake due to obsession with food, and impaired physical activity.[3, 9] In the long term these metabolic side effects may contribute to the higher risk of childhood leukemia survivors to develop metabolic syndrome (MetS).[10-13] A recent study in 784 ALL survivors showed that 34% has MetS, as defined by National Cholesterol Education Program – Adult Treatment Panel III criteria.[14]

Although prednisolone also affects metabolism, dexamethasone has a more intense toxicity profile[2, 15] with major metabolic changes in a short period of time, like increased energy intake and insulin resistance. Dexamethasone-induced insulin resistance after only five days has been reported in a study in 18 pediatric ALL patients.[6] So, even in short term dexamethasone seems to affect components of the MetS. The latter is defined for children ≥10 years as abdominal obesity, plus any two or more of the clinical features: hyperglycemia, hypertriglyceridemia, reduced HDL cholesterol, hypertension.[16] Esbenshade *et al.* longitudinally followed 34 patients for one year of maintenance therapy, containing prednisone or dexamethasone, and documented a worsening of weight gain, insulin resistance, and leptin levels in the same children with ALL over the year.[17] This emphasizes the impact of acute glucocorticoid-induced metabolic toxicity on the long term. Since dexamethasone pulses are administered in the Dutch Childhood Oncology Group (DCOG) ALL protocols for one and a half year during maintenance, it is conceivable that risk factors for developing MetS accumulate. However, prospective studies on direct dexamethasone-induced effects on all components of the MetS in a substantial cohort of pediatric ALL patients are lacking. In addition no information is available on the role of dexamethasone pharmacokinetics on the occurrence of MetS. We therefore in the context of a randomized controlled trial, studied prospectively the acute effects of dexamethasone on all components of the MetS, and the role of dexamethasone serum levels in a subset of patients.

MATERIALS AND METHODS

Patients

The study protocol was approved by the Ethics committee of the Erasmus MC and the local ethics committees at each participating center (MEC-2012-155/ EudraCT 2011-003815-46): University Medical Center Utrecht, Academic Medical Center Amsterdam, VU Medical Center, University of Groningen Medical Center. In accordance with national regulations, parents and legal guardians of patients provided written informed consent; patients 12-16 years of age also provided their own written informed consent. Fifty children with ALL, aged 3 to 16 years, who completed the placebo course of the multicenter randomized controlled trial, the Dexadays study (NTR3280)[18], were included for the present study.

Patients received dexamethasone pulses during maintenance phase according to DCOG ALL protocols in five pediatric oncology departments in the Netherlands. Since asparaginase treatment may cause hypoalbuminemia, is associated with greater plasma exposure to dexamethasone[19], influences glucose metabolism, and may cause changes in lipid levels, we included children only after discontinuation of the asparaginase phase. Patients underwent an oral dexamethasone course of 5 days (6 mg/m^2/day) with addition of a placebo (10 mg/m^2/day)(containing 0.26 g/mL sorbitol 70% solution) during the Dexadays study. (Figure 1) Dexamethasone was given together with vincristine 2

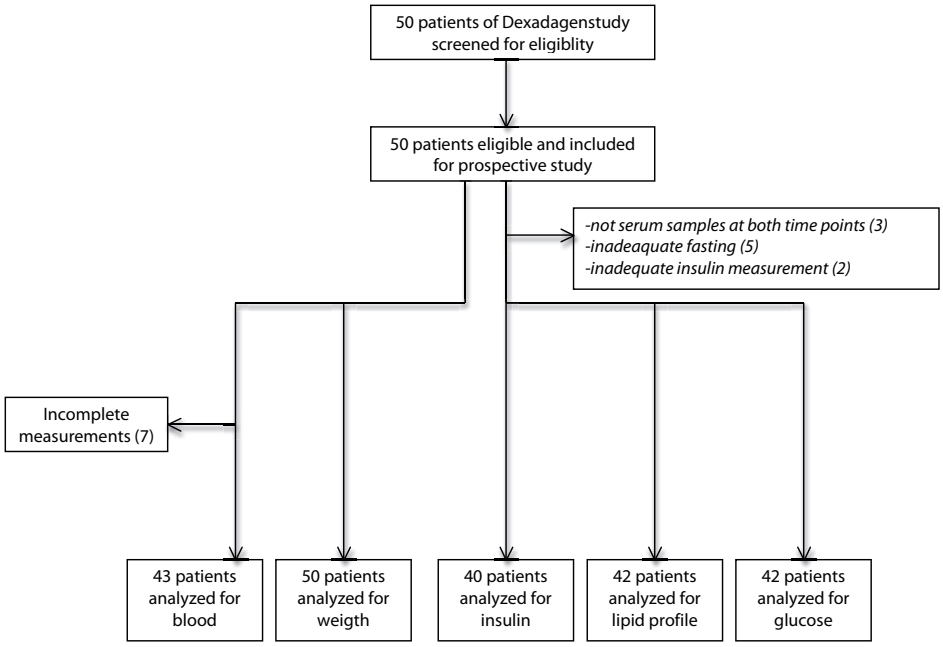

Figure 1. *Consort flowchart.*

mg/m^2 on day 1, 6-mercaptopurine 50 mg/m^2 daily and methotrexate 30 mg/m^2 weekly. Our primary outcome was metabolic toxicity during one dexamethasone course.

Anthropometry

Height (m), weight (SDS), waist circumference (cm) were measured before start of dexamethasone (T1) at baseline. Blood pressure (mmHg) was measured at T1 and at the morning of the fifth dexamethasone course day (T2). Blood pressure was measured with the subject in sitting position after one hour of rest on the right arm with the Dinamap® Procare. Cut-off values for these anthropometric parameters corrected for age and sex were obtained from Cole *et al.*[20] (BMI: adiposity, obesity), the reference values of the National High Blood Pressure Education Program Working Group[21] (hypertension), and Fredriks *et al.*[22] (waist circumference: >1.3 SD = abdominal adiposity, >2.3 SD = abdominal obesity). Blood pressure was expressed as SDS adjusted for height and sex.[23] Hypertension was defined as blood pressure (either systolic or diastolic) above the 95th percentile for age and sex.[21]

Laboratory measurements

At T1 and T2, following overnight fasting, peripheral blood samples were obtained for measurement of insulin, glucose, total cholesterol, high-density lipoprotein–cholesterol (HDL), low-density lipoprotein-cholesterol (LDL) and triglycerides levels. Laboratory reference values of the department of Clinical Chemistry of the Erasmus Medical Center[24] and International Diabetes Federation[16] were used. The serum samples were stored at -80^{o}C until the analyses were performed after inclusion was completed. Serum samples were determined in one and the same laboratory using an enzymatic *in vitro* assay (Roche Diagnostics, Mannheim, Germany).[18]

Impaired fasting glucose (IFG) was defined as fasting glucose ≥5.6 mmol/L and <7.0 mmol/L using the ADA criteria.[25] Hyperinsulinemia was defined as a fasting insulin value: pre-pubertal (≥15 mU/L), mid-puberty (≥30 mU/L), and post-pubertal (≥20 mU/L).[26] Insulin resistance index was calculated by homeostasis model assessment of insulin resistance (HOMA-IR), which is calculated by the following formula: fasting glucose (mmol/L)* fasting insulin (mU/L) / 22.5.[27] A cut-off of HOMA-IR ≥ 3.4 is chosen because of its predictive value for impaired glucose tolerance and diabetes mellitus, based on literature.[28-33] We also performed the analysis using the cut-off value HOMA-IR>4.39 for insulin resistance as used by Chow *et al.*[6], in order to be able to compare our data.

Metabolic syndrome (MetS) was defined by the International Diabetes Federation (IDF) criteria for children ≥10 years of age with abdominal obesity (waist circumference ≥90 percentile), plus any two of more of the clinical features: hyperglycemia, hypertriglyceridemia, reduced HDL cholesterol, hypertension.[16]

Pharmacokinetics

Dexamethasone trough levels were measured at T2 (Pharmacology department, Academic Medical Center, Amsterdam). Due to the lack of available plasma samples, serum samples that were obtained on the morning of the fifth day before dexamethasone administration were used. No correction for measurement in serum was needed, since serum levels did not significantly differ from plasma levels. (*P*=0.37) The exact time of oral dexamethasone intake the night before was retrieved from patient diaries.

Statistics

The effect of dexamethasone was assessed by comparing the results at baseline and at day 5 by a Paired T-test (for normally distributed measures) or a Wilcoxon Signed Rank test. Pearson's coefficient and Spearman's coefficient described the correlation (r) between values. All analyses were performed using SPSS, version 21.

RESULTS

Baseline characteristics

For this study, 50 patients were included (46% males). (Figure1) Serum samples for laboratory measurements were missing for 3 patients. The median age was 6.0 years (interquartile range (IQR): 4.0, 10.3). Adiposity, based on BMI, was present in 7% of these patients at diagnosis and increased to a prevalence of 19% at study baseline. Abdominal adiposity, based on waist circumference >1.3 SD, was found in 48% of the patients at study baseline. Obesity, defined by BMI, was not present at diagnosis and increased to a prevalence of 4% at study baseline. Abdominal obesity, based on waist circumference >2.3 SD, was present in 10% at study baseline. Weight SDS (r=0.44, *P*=0.00) and the presence of a high BMI (r=0.35, *P*=0.02) were linearly correlated with age.

Glucose metabolism

Serum samples of five patients were excluded for measurement because of inadequate fasting. Median fasting glucose levels (*N*=42) significantly increased (Figure 2) after four days of dexamethasone treatment (median: 0.40 mmol/L (IQR: -0.20, 1.03), *P*=0.00). An impaired fasting glucose (IFG)[27] was not observed at baseline. Two patients (5%) had an IFG after four days of dexamethasone (*P*=0.32). (Table 1) Higher fasting glucose levels on dexamethasone were correlated with older age (r=0.49, *P*=0.03). There was no influence of gender or week of maintenance phase on glucose levels.

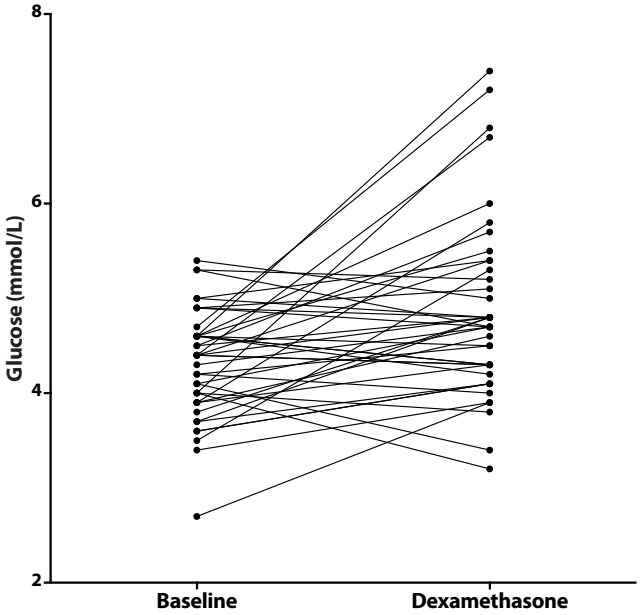

Figure 2. *Fasting glucose values at baseline and after four full days of dexamethasone treatment. N=42.*

Table 1. *Increase (=delta) in fasting lipid, glucose and insulin levels (median (Inter Quartile Range)) or the increase in occurrence of high glucose, HDL, LDL, total cholesterol, triglycerides or insulin levels during 4 days of dexamethasone treatment. The effect of dexamethasone on lipids and glucose (N=42) was tested with a Paired T-test. The effect of dexamethasone on insulin (N=40) and HOMA-IR (N=39) was tested with a Wilcoxon Signed Rank test.*

	Delta	P-value
Glucose (mmol/L) Median (IQR)	0.40 (-0.20, 1.03)	0.00
Hyperglycemia N(%)	2 (5)	0.96
HDL Median (IQR)	0.11 (-0.06, 0.32)	0.00
Low HDL N(%)	-2 (5)	0.01
High HDL N(%)	6 (14)	
LDL Median (IQR)	0.24 (-0.03, 0.52)	0.00
High LDL N(%)	4 (9)	0.00
Total Cholesterol Median (IQR)	0.40 (0.08, 0.73)	0.00
High Total Cholesterol N(%)	7 (16)	0.01
Triglycerides Median (IQR)	0.17 (-0.08, 0.47)	0.04
Hypertriglyceridemia N(%)	7 (17)	0.09
Insulin (pmol/L) Median (IQR)	173.75 (129.28, 334.55)	0.00
High Insulin N(%)	32 (80)	0.00
HOMA-IR Median (IQR)	4.66 (3.50, 10.27)	0.00
Insulin resistance HOMA-IR > 4.39 N(%)	26 (67)	0.00
Insulin resistance HOMA-IR>3.4 N(%)	30 (77)	0.00

Laboratory measurements

The median insulin level at baseline (*N*=40) was 25.2 pmol/L (IQR: 14.4, 73.4). Four days of dexamethasone significantly increased insulin levels (median: 173.75 pmol/L (IQR: 129.28, 334.55), *P*=0.00). (Figure 3) Hyperinsulinemia was present in 4 patients (10%) at baseline and in 36 patients (90%) after 4 days of dexamethasone treatment (*P*=0.00). HOMA-IR (*N*=39) values significantly increased over the course (median: 4.66 (IQR: 3.50, 10.27), *P*=0.00, Figure 4). The prevalence of insulin resistance (HOMA-IR >3.4) significantly increased on dexamethasone (8% at T1 vs 85% at T2, *P*=0.00). Even using the cut-off values as defined by Chow *et al.* (HOMA-IR > 4.39)[6], insulin resistance significantly increased from 5% to 72% (*P*=0.00). Hyperinsulinemia at baseline was correlated with older age (r=0.41, *P*=0.01). None of the patients received insulin therapy during the dexamethasone course. Gender and the week of maintenance phase did not influence insulin resistance.

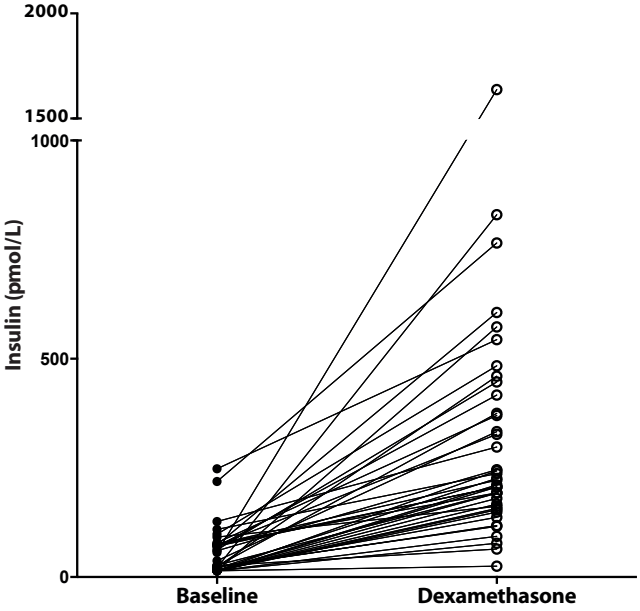

Figure 3. *Insulin (pmol/L) values at baseline and after four full days of dexamethasone treatment. N=40.*

Lipid profile

Serum samples of five patients were excluded for measurement because of inadequate fasting. Dexamethasone increased median fasting blood levels (*N*=42) of HDL (median delta: 0.11 mmol/L (IQR: -0.06, 0.32), *P*=0.00), LDL (median: 0.24 mmol/L (IQR: -0.03, 0.52), *P*=0.00), total cholesterol (median: 0.40 mmol/L (IQR: 0.08, 0.73), *P*=0.00), and triglycerides (median: 0.17 mmol/L (IQR: -0.08, 0.47), *P*=0.04) (Figure 5, Table 1). High

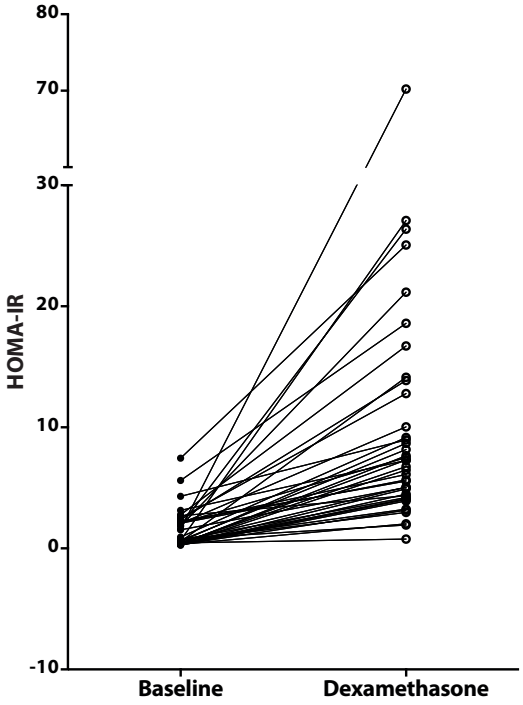

Figure 4. *HOMA-IR values at baseline and after four full days of dexamethasone treatment. N=39.*

HDL, high LDL, high total cholesterol and hypertriglyceridemia were already present in respectively 12%, 17%, 10% and 24% at baseline, and increased to 26%, 26%, 26% and 41% respectively by dexamethasone. (Table 1) Dexamethasone administration did not result in a decrease of HDL. Gender, age, and the week of maintenance phase did not influence dexamethasone-induced change in lipid serum levels.

Blood pressure

Blood pressure was successfully measured for 43 patients. Dexamethasone treatment significantly increased diastolic blood pressure SDS (median: 0.33 (IQR: -0.24, 0.94), $P<0.01$), and systolic blood pressure SDS (median: 0.58 (IQR: -0.61, 1.24), $P<0.05$). (Figure 6) Increase in diastolic blood pressure was inversely related with age (r=-0.43, $P<0.01$).

Hypertension (either diastolic or systolic), defined as a blood pressure >95th percentile corrected for age and sex, was present in 25% of the patients at baseline and increased to 39% by dexamethasone ($P=0.14$), and was inversely correlated with age (r=-0.36, $P=0.02$). Systolic hypertension at baseline was inversely correlated with age (r=-0.33, $P=0.03$) and more often diastolic hypertension on dexamethasone (r=-0.47, $P=0.00$).

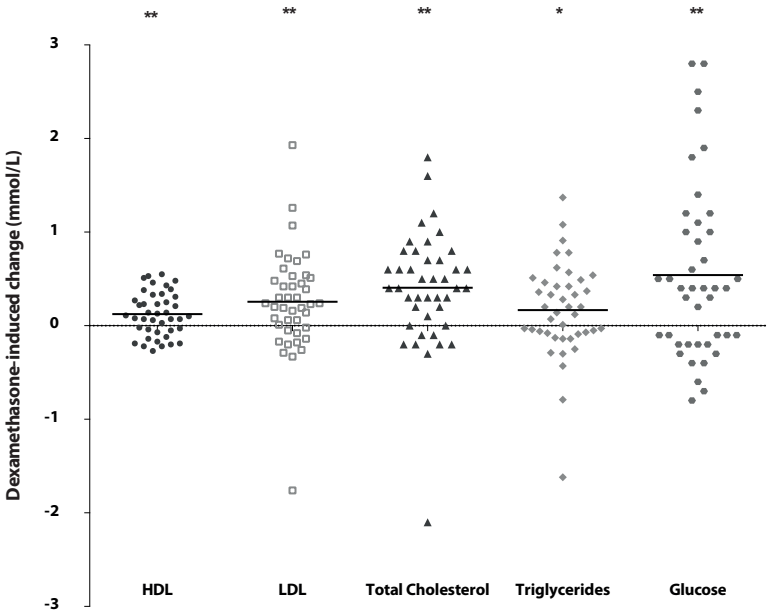

Figure 5. *Dexamethasone-induced changes in HDL, LDL, total cholesterol, triglyceride and glucose values (mmol/L) in four days: value day 5 - value baseline. The effect of dexamethasone was tested with a Paired T-test. *= P<0.05. **= P<0.01. Median is depicted for each laboratory measurement. A positive score reflects an increased level by dexamethasone.*

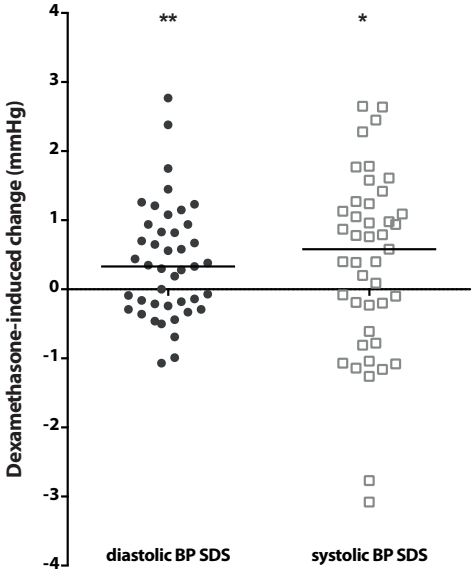

Figure 6. *Dexamethasone-induced changes in diastolic and systolic blood pressure (BP) SDS values by four full days of dexamethasone treatment: value day 5 - value baseline. N=43. Median is depicted. A positive score reflects an increased SDS level by dexamethasone. *= P<0.05. **= P<0.01.*

Components of the metabolic syndrome

Table 2. *The presence of components of the metabolic syndrome (MetS) at baseline and after four full days of dexamethasone treatment.*

	Abdominal obesity	Hyper-glycemia	Hyper-triglyceridemia	Reduced HDL cholesterol	Hypertension	Abdominal obesity + ≥2 MetS components
Baseline N (%)	5 (10)	0 (0)	10 (24)	2 (5)	11 (25)	0 (0)
On Dex N (%)	7 (15)	2 (5)	17 (41)	0 (0)	17 (39)	3 (7)

Components of the MetS

The International Diabetes Federation defines MetS for children >10 years as abdominal obesity, plus any of the two or more of the following clinical features: hyperglycemia, hypertriglyceridemia, reduced HDL cholesterol, and hypertension. Abdominal obesity was observed in 7 patients (15%, 3 males) after four days of dexamethasone. These children had a median age of 9.0 years (IQR: 3.0, 12.0). Three of these patients had developed hyperglycemia, hypertension, or hypertriglyceridemia, which are all components of the MetS, during four days of dexamethasone treatment. (Table 2) The oldest patient was 12 years of age and had a fasting glucose of ≥5.6 mmol/L and a high systolic blood pressure at T2. The other patients were younger than 10 years of age and developed a high blood pressure and hypertriglyceridemia within four days of dexamethasone treatment.

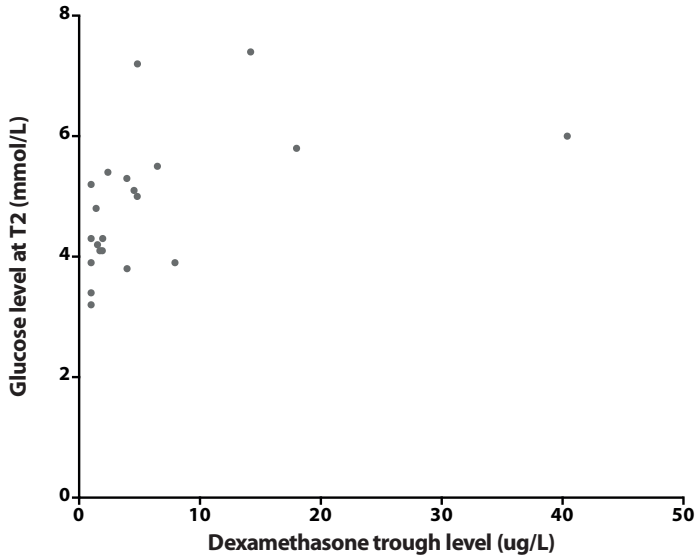

Figure 7. *Dexamethasone trough serum levels were positively correlated with glucose levels after four days of dexamethasone treatment (r=0.63, P<0.01). The correlation was described with a Spearman's coefficient.*

Pharmacokinetics

Dexamethasone trough levels after four days of treatment were available for 24 patients, and showed a median of 3.97 ug/L (IQR: 1.45 – 11.81 ug/L). Trough levels were not correlated with age, gender or week of maintenance phase. Dexamethasone trough levels were not significantly different between patients with or without abdominal obesity (P=0.17).

Dexamethasone trough levels were positively correlated with increase in glucose levels during dexamethasone (r=0.66, P<0.01) and with glucose levels after four days of dexamethasone treatment (r=0.63, P<0.01, Figure 7). Dexamethasone trough levels did not directly influence insulin resistance, lipid serum levels and blood pressures.

DISCUSSION

This is the first study that investigated the acute effects of four days of dexamethasone on all components of the metabolic syndrome in a substantial cohort of ALL patients. We showed that one dexamethasone course significantly increased glucose, lipids, insulin resistance, and blood pressure, thereby influencing three out of six factors of the MetS; hyperglycemia, hypertriglyceridemia, and hypertension. In contrast to Chow *et al*[6], who reported an 35.5% increase (N=31) in insulin resistance during one steroid (prednisone/dexamethasone) course, we found an dexamethasone-specific increase in insulin resistance of 67% in a larger population. This could be explained by the fact that 6 mg/m^2 dexamethasone is more potent than 40 mg/m^2 prednisolone.[1] The amount of preceding dexamethasone courses did not influence the metabolic effects of dexamethasone.

On the short-term these metabolic toxicities could have various consequences. High glucose levels and insulin resistance could induce microvascular problems[34], impairment of the innate immune system[35], and changes in hemostatic responses[36]. The short-term metabolic toxicities are not age-dependent. Our results emphasize, that although MetS criteria are lacking for children <10 years, young ALL patients are not spared from metabolic toxicity.

On the long term the accumulating disguised metabolic toxicity of dexamethasone courses during one and a half year of maintenance therapy may contribute to the high incidence of MetS in childhood leukemia survivors[14, 37], thereby increasing the risk for diabetes and cardiovascular diseases.[14] Esbenshade *et al.* has already reported an increase in insulin resistance of 49% during one year of maintenance phase containing glucocorticoids [17], which supports the hypothesis of accumulating metabolic toxicity. In comparison, inadequate glucose control in pediatric diabetes mellitus type I has also shown to lead to more long-term complications.[38]

Dexamethasone-induced weight gain, which is influenced by changes in eating behavior[3], may contribute to the cardiovascular and diabetic risk.[3, 17] The prevalence of adiposity (based on BMI) in our population increased substantially from 7% at diagnosis to 19% at study baseline. The study baseline prevalence of adiposity was higher compared to the Dutch general population (13%)[39], but the incidence of obesity was comparable (4%)[39]. However, abdominal adiposity and abdominal obesity, both common steroid-related side effects[40], were significantly more common in our population. Measuring waist circumference may therefore be valuable, since steroids induce redistribution of fat to the upper trunk and face, with a loss of fat in the extremities.[40]

One of the other metabolic side effects is dexamethasone-induced hypertension, which has been described before during 28 days of prednisone-based induction therapy in 45% of ALL patients[7], of whom 27% was receiving anti-hypertensive drugs. Hypertension was seen in 6% of our ALL patients during five days of dexamethasone therapy, and no anti-hypertensive drugs were administered. Dexamethasone-induced hypertension could involve the promotion of inotropic and vasoconstrictive effects on the cardiovascular system.[40] Our study confirmed earlier findings[7] that young age seems to be a risk factor for developing steroid-induced hypertension.

Dexamethasone trough levels were positively correlated with glucose levels. These increased glucose levels could be caused by inhibition of glucose uptake in peripheral tissues and stimulation of hepatic gluconeogenesis by direct effect on hepatic gene expression.[40] We presumed more metabolic parameters to be associated with dexamethasone clearance, since toxicity has been associated high with dexamethasone levels before.[41] The lack of influence of dexamethasone levels on lipids and insulin could be caused by insufficient power and short-term exposure. Also, the pathophysiologic mechanism of metabolic side effects, although it has not been completely unraveled, may be side effect specific[40, 42], which could possibly explain the differences between metabolic side effects. The results underscore the need for a large pharmacokinetic study with long follow-up to further investigate the association between dexamethasone drug levels and metabolic toxicity. Since dexamethasone trough levels varied widely between patients, individualized dosing of dexamethasone in children with ALL may be the next step.

To conclude, we demonstrated the serious disguised metabolic toxicities of dexamethasone on short-term. We showed that even four days of dexamethasone in ALL patients significantly affects components of the MetS, and that dexamethasone levels influence glucose metabolism. Our findings do not have direct clinical consequences, but they could form the basis for further studies on personalized treatment, for example individualized dexamethasone dosing, targeted therapy with selective glucocorticoids[43],

and programs supporting a healthy diet and physical activity. This is important because the accumulating disguised metabolic toxicities, together with the weight gain during dexamethasone treatment, may contribute to the higher prevalence of MetS and cardiovascular risk childhood leukemia survivors.

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Chapter 6

Predicting the neuropsychological side effects of dexamethasone in pediatric acute lymphoblastic leukemia.

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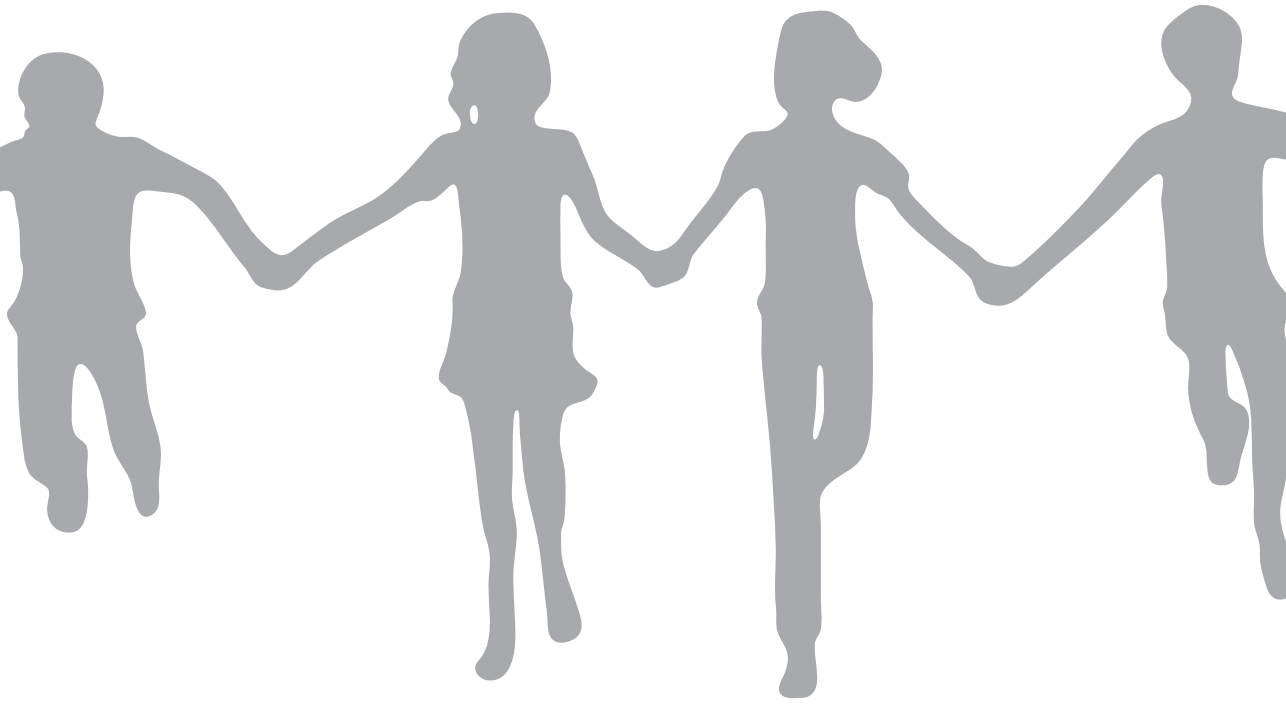

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ABSTRACT

Dexamethasone, an effective treatment of acute lymphoblastic leukemia (ALL), can induce serious neuropsychological side effects that vary between patients. We hypothesized that these side effects are influenced by glucocorticoid sensitivity at the tissue level. We therefore prospectively studied whether we could predict these side effects by a very low dose dexamethasone suppression test (DST) or by dexamethasone trough levels. Fifty ALL patients (3-16 years) were included during the maintenance phase (with dexamethasone courses) of the Dutch ALL protocol. As marker of glucocorticoid sensitivity, the salivary very low dose DST was used. A post-dexamethasone cortisol <2.0nmol/L was considered a hypersensitive response. Neuropsychological endpoints consisted of questionnaires on psychosocial and sleeping problems before and during a dexamethasone course. Dexamethasone trough levels were measured during dexamethasone (6mg/m^2) treatment.

Patients with a hypersensitive response (N=13, 26%) had more dexamethasone-induced behavioral problems (median delta: 1.0 (inter quartile range: 0.0,2.0) versus 0.0 (-0.5,1.0), *P*=0.01), sleeping problems (4.5 (0.0, 13.5) versus 0.0 (-3.0, 2.0), P=0.03), and/or somnolence (3.0 (1.0, 6.0) versus 1.0 (-0.5, 2.5), P<0.05). The positive predictive value of the DST for psychosocial problems and sleeping problems was 50% and 30% respectively. Dexamethasone levels were not associated with neuropsychological side effects.

We conclude that the very low dose DST and dexamethasone trough levels could not accurately predict neuropsychological side effects. However, patients with glucocorticoid hypersensitivity experienced significantly more dexamethasone-induced depressive symptoms. Future studies should further elucidate the glucocorticoid sensitivity dependent mechanisms by which neuropsychological side effects are influenced.

INTRODUCTION

Dexamethasone is an important drug in the treatment of pediatric acute lymphoblastic leukemia (ALL)[1-3] but its side effects can have major impact on the quality of life of patients.[4-6] Currently, in the Dutch ALL protocols, during the maintenance period of 1.5 years, medium risk ALL patients receive five-day standard-dose dexamethasone courses in three weekly cycles. The reported occurrence of neuropsychological side effects in pediatric ALL patients shows extreme variation of 5-75%.[4, 7, 8] In a minority of cases side effects are even so severe that dexamethasone treatment is switched to prednisolone or discontinued, accepting a negative impact on outcome. Identification of patients at risk for developing neuropsychological side effects would enable individualized treatment to potentially reduce dexamethasone-induced side effects. Still, tools to predict neuropsychological side effects during dexamethasone treatment are lacking. Corticosteroids bind to mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs). The imbalance in activation of the GR and the MR in the brain caused by exogenous glucocorticoids, seem to play an important role in the pathophysiology of neuropsychological side effects.[9] Previous studies indicate that although the sensitivity of the GR seems variable between individuals, the intra-person sensitivity is stable. This is supported by the observation that baseline plasma cortisol concentrations, which are regulated by a GR dependent feedback system, vary widely between normal subjects but show high intra-individual stability.[10]

The sensitivity of the hypothalamic-pituitary-adrenal (HPA) axis feedback system to glucocorticoids can be measured by a very low dose dexamethasone (0.25 mg) suppression test (DST). In population-based studies, subjects with the highest baseline cortisol concentrations also had the highest post-DST concentrations. It further seems that within an individual, there is a specific set point for HPA-activity, which can be defined before as well as after a low dose of dexamethasone. A dose of 0.25 mg dexamethasone, which results in a subtotal suppression of cortisol levels, does not influence this set point. In the 0.25 mg DST, post-DST cortisol levels show a Gaussian distribution, where the subjects in the extremes are relatively hypersensitive or resistant to glucocorticoids.[10]

Apart from differences in glucocorticoid sensitivity, pharmacokinetic variation is also a possible explanation for inter-individual differences in dexamethasone related side effects. Dexamethasone clearance in pediatric ALL patients is inversely related to age.[11] Poor dexamethasone clearance has been reported in ALL patients with osteonecrosis, another serious side effect of dexamethasone.[11] Whether dexamethasone pharmacokinetics also influences the occurrence of neuropsychological side effects, is unknown.

The aim of our study was to assess the predictive value of the very low dose dexamethasone suppression test, and to assess whether dexamethasone serum levels influ-

ence the occurrence of neuropsychological side effects of dexamethasone in pediatric ALL.

MATERIAL AND METHODS

Participants

Children with ALL, aged 3 to 16 years, who were enrolled in the multicenter randomized controlled trial, the Dexadays study (NTR3280)[12], were included. Patients were receiving 5-day dexamethasone pulses (6mg/m^2/day) every three weeks during maintenance phase according to the DCOG ALL protocols. Measurements were performed before and during the placebo course of the Dexadays study. The study protocol was approved by the local ethics committee (MEC-2012-155/EudraCT 2011-003815-46). The study was performed in accordance with the Declaration of Helsinki and followed the principles of good clinical practice.

Cortisol measurement

A very low dose salivary dexamethasone suppression test (DST) was performed in the week before a dexamethasone pulse. Salivary cortisol measurement is a reliable and minimally invasive method to assess the active, unbound form of cortisol.[13] Patients were requested to collect saliva samples at home using Salivette sampling devices (Sarstedt, Rommelsdorf, Germany) after obtaining informed consent and detailed oral and written instructions concerning the saliva sampling. Saliva samples were collected in the week before a dexamethasone course. Five saliva samples were collected during two consecutive days at five time points (Table 1); that is, immediately at awakening (T1= baseline), at noon (T2), around 4pm (T3), in the evening at bedtime (T4), and immediately at awakening on the consecutive day post-DST (T5). After obtaining the T4 sample on the first day, a very low dose of dexamethasone (0.25 mg/ 1.73 m^2) was taken orally. Parents were asked to write down the exact times and date of saliva collections. Furthermore, patients were instructed not to brush their teeth and not to eat 15 minutes before saliva sampling to avoid contamination of saliva with blood caused by micro-injuries to the oral cavity. Besides these restrictions, the children were otherwise free to follow their normal daily routines on the sampling day. Parents were instructed to store saliva samples in the fridge until finalizing the collection of five samples. Thereafter, the samples were sent to the Diagnostic Endocrinology Laboratory of the Erasmus MC, where samples were stored in the freezer at -80°C until completion of the inclusion. Cortisol concentrations were measured using a commercial immunoassay with chemiluminescence detection (CLIA; IBL Hamburg, Hamburg, Germany). The lower limit of detection was 0.4 nmol/ liter. (Erasmus MC. 2015) Data were screened for quality of cortisol measurements. Saliva

Dexamethasone Suppression Test scheme

Table 1. *Schedule of the very low dose dexamethasone suppression test.*

Time	*Day 1*	*Day 2*
Morning	Fasting saliva sample (T1)	Fasting saliva sample (T5)
Noon	Saliva sample (T2)	
Afternoon	Saliva sample (T3)	
Evening	Saliva sample (T4)	
Evening	Very lose dose dexamethasone	

samples were used to measure the cortisol day curve (T1-4), and the post-DST cortisol level (T5). HPA-axis response was assessed by comparing morning cortisol levels at T1 and T5 (cortisol suppression).

Severe cortisol suppression was defined as a post-DST cortisol level (T5) < 2.0 nmol/L. This cut-off was chosen based on the Guidelines for Cushing Syndrome of the Endocrine Society, which recommends a cut-off for suppression of the post-DST (1mg DST) serum cortisol levels of <50 nmol/L to achieve high sensitivity rates.[14] Since serum cortisol levels are almost 27 times higher than salivary cortisol levels[15], we chose 2.0 nmol/L as cut-off level for severe post-DST cortisol suppression.

Dexamethasone pharmacokinetics

Dexamethasone trough levels were measured after four full days of 6 mg/m^2 dexamethasone (Pharmacology department, Academic Medical Center, Amsterdam). For this purpose, serum samples, obtained on the morning of the fifth day before dexamethasone administration, were used. Time of intake of dexamethasone the night before was retrieved from patient diaries.

Neuropsychological side effects

Psychosocial problems were measured by the parent-reported Strengths and Difficulties Questionnaire in Dutch (SDQ-Dut)[16] before start of dexamethasone and at day 5 of the dexamethasone course (placebo). A delta-score (i.e., the difference between two scores) was calculated by subtracting the score on treatment day 1 score from the score on treatment day 5.[12] The SDQ-Dut has 25 items on 5 subscales: Emotional symptoms, Conduct problems, Hyperactivity and inattention, Peer relationship problems, and Prosocial behavior. The cumulative score was calculated by combining the 5 subscales minus the Prosocial behavior scale into a "Total difficulties" score. The Impact score is an extra subscore and reflects the impact of the difficulties on the child's life. Clinically relevant psychosocial problems were defined as a delta SDQ Total difficulties score ≥5.[12]

Sleeping problems were assessed by the Sleep Disturbance Scale for Children (SDSC)[17] on the first day and fifth day of the dexamethasone course. It has a Total score and covers

6 most common sleep disorders of childhood: Disorders of Initiating and Maintaining Sleep (DIMS), Sleep Breathing Disorders (SBD), Disorders of Arousal (DA), Sleep Wake Transition Disorders (SWTD), Disorders of Excessive Somnolence (DES), and Sleep Hyperhydrosis (SHY).[12] Clinically relevant sleeping problems were defined as a delta SDSC Total score ≥7.[12]

Statistics

Cortisol values were compared by a Paired T-test (for normally distributed measures) or a Wilcoxon Signed Rank test. Spearman's coefficient described the correlation (r) between cortisol values and SDQ scores and SDSC scores. All analyses were performed using SPSS, version 21. To evaluate the predictive value of the salivary low dose DST on dexamethasone-related neuropsychological side effects, a nested analysis was performed. The positive predictive value (PPV) is defined as (number of true positives based on SDQ≥5 or SDSC≥7) / (number of positives based on DST< 2.0 nmol/L). The probability value used to identify significance was $P<0.05$.

RESULTS

Baseline characteristics

48 of 50 patients, included in the Dexadays study[12], completed the salivary very low dose suppression test. Patients (46% male) had a median age of 6.0 years (IQR: 4.0, 10.3). The post-DST cortisol level (T5) was missing in one patient.

Adrenal function during maintenance

Baseline cortisol levels (T1) varied widely between patients (median 11.2 nmol/L, (IQR: 7.3, 15.4)). Seventeen patients (35%) revealed a morning cortisol level below the reference range (<9.0 nmol/L)[18] at T1. Cortisol levels decreased during the day (T2: median 3.6 (IQR: 2.7, 5.4), T3: median 2.6 (IQR: 1.7, 3.5), T4: median 1.4 (IQR: 0.8, 1.9), reflecting the physiological circadian rhythm of cortisol production. (Figure 1) Morning cortisol levels were higher in female patients (female: median 13.1 nmol/L (IQR: 10.8, 17.5) versus male: median 8.2 nmol/L (IQR: 6.0, 12.0), $P=0.01$). No associations of morning cortisol levels (T1) with age, week of maintenance phase or dexamethasone trough level were found.

The post-DST cortisol levels (T5) were significantly lower than baseline cortisol levels at T1, respectively median 3.7 (IQR: 1.9, 7.9) versus median: 11.2 (IQR: 7.3, 15.4) ($P<0.001$), and varied widely between patients. The median suppression in cortisol level following a very low dose of dexamethasone was 6.8 nmol/L (IQR: -9.6, -1.0). Seven patients (15%) did *not* show dexamethasone-induced cortisol suppression. Dexamethasone-induced cortisol suppression was not associated with gender, age nor with week of maintenance.

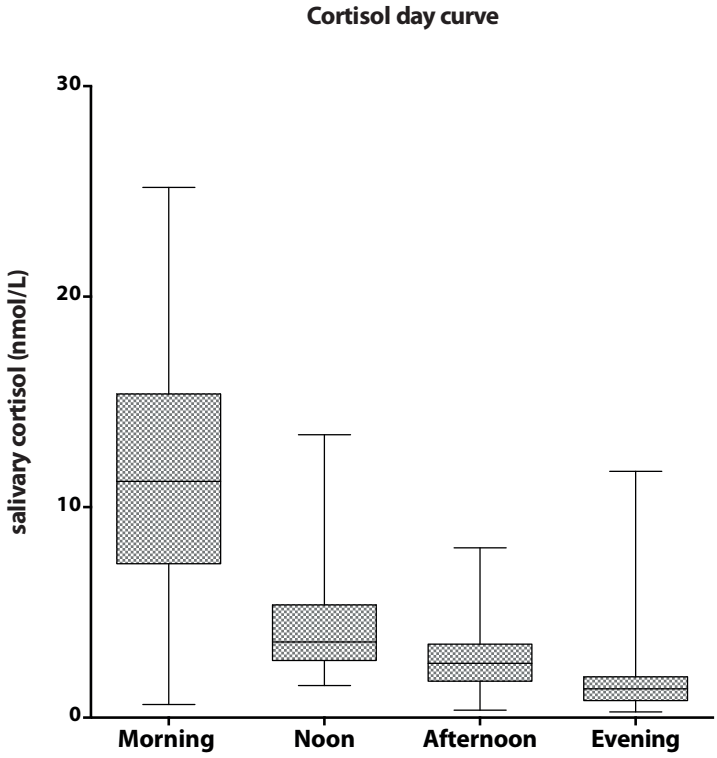

Figure 1. *Cortisol day curve. The median cortisol level (nmol/L) of the study population at four time points is depicted.*

HPA-axis reactivity as predictor of neuropsychological side effects

Increased cortisol suppression was correlated with a higher parent-reported SDQ Impact score after four days of dexamethasone treatment (r=-0.43, *P*<0.01). In the total group more pronounced cortisol suppression (defined as a post-dexamethasone cortisol level <9.0 nmol/L) was associated with more increase in Conduct problems (r=-0.35, *P*=0.02) and Impact of difficulties (r=-0.34, *P*=0.03) during dexamethasone treatment. Cortisol suppression was not associated with an increase in other SDQ subscales or SDSC subscales on dexamethasone.

Thirteen patients (26%) had severely suppressed post-DST cortisol levels below 2.0 nmol/L[14]. These children had significantly more dexamethasone-induced Conduct problems than patients with a post-DST cortisol level ≥2.0 nmol/L (median delta SDQ Conduct problems: 1.0 (IQR: 0.0, 2.0) vs 0.0 (IQR: -0.5, 1.0), *P*=0.01). (Figure 2) The difference in delta score between two groups was 0.7 standard deviation score on the Conduct problems subscale. This subset of patients also had significantly more dexamethasone-induced Total sleeping problems (delta 4.5 (IQR: 0.0, 13.5) vs 0.0 (IQR: -3.0, 2.0), P=0.03),

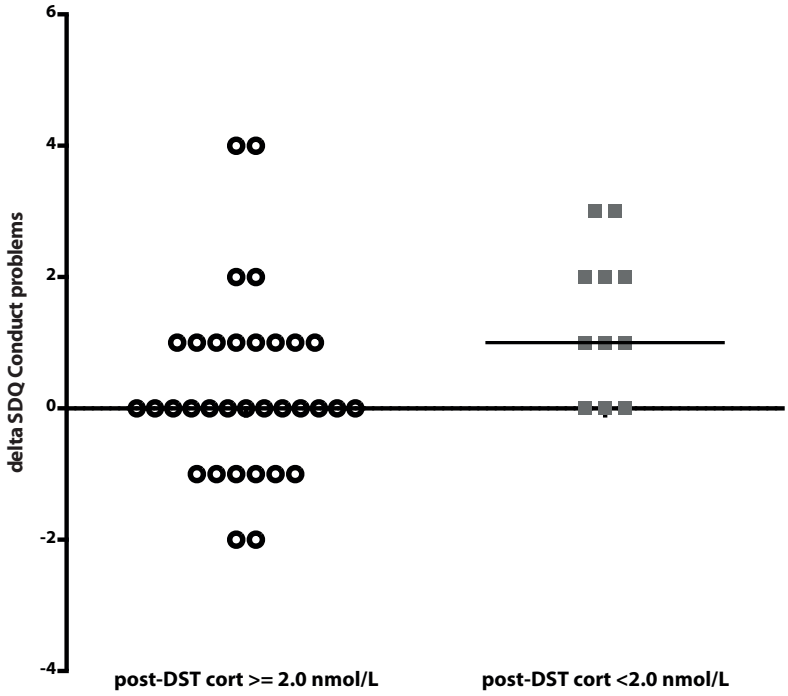

Figure 2. *Patients with a post-DST cortisol <2.0 nmol/L (N=13) had significantly more dexamethasone-induced Conduct problems. Medians are depicted.*

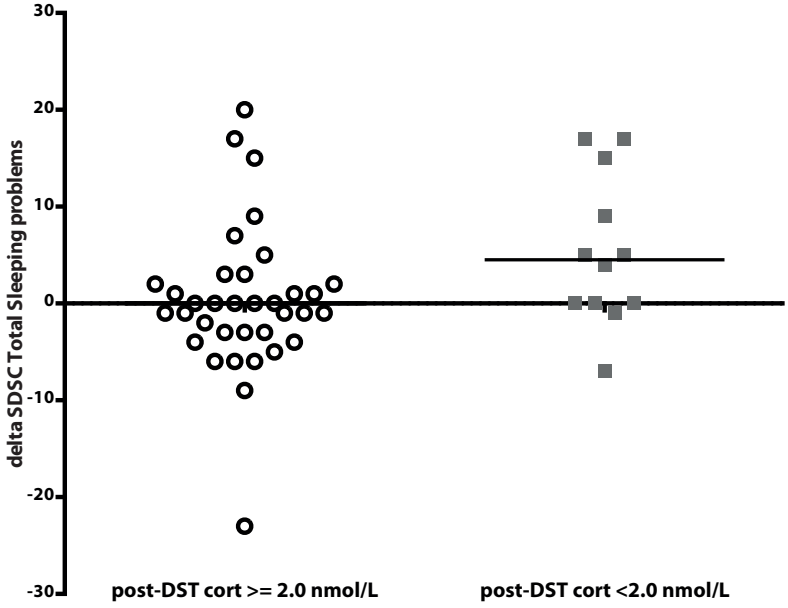

Figure 3. *Patients with a post-DST cortisol <2.0 nmol/L (N=13) had significantly more dexamethasone-induced Total sleeping problems.*

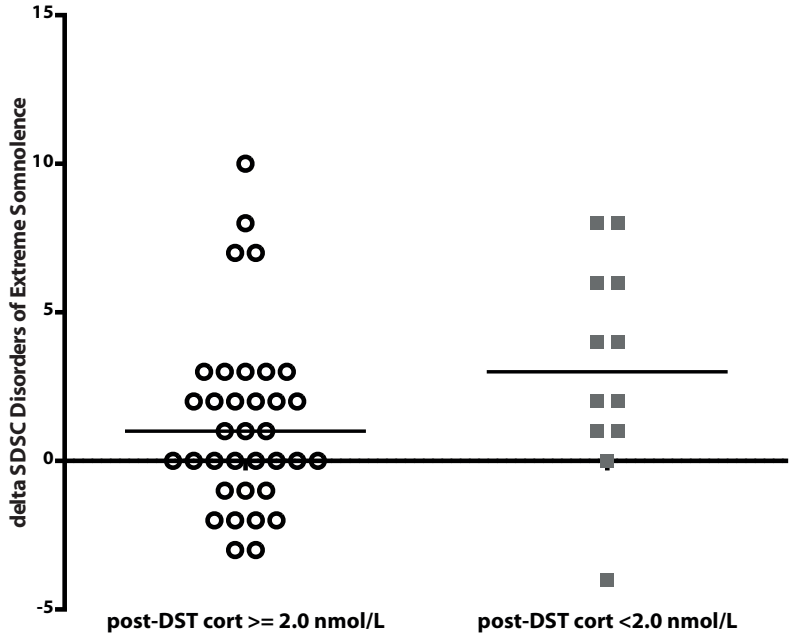

Figure 4. *Patients with a post-DST cortisol <2.0 nmol/L (N=13) had significantly more dexamethasone-induced Disorders of Excessive Somnolence.*

and Disorders of Excessive Somnolence (delta 3.0 (IQR: 1.0, 6.0) vs 1.0 (IQR: -0.5, 2.5), P<0.05). (Figure 3-4)

Although patients with glucocorticoid hypersensitivity had more dexamethasone-induced conduct problems and sleeping problems, the positive predictive value of severe cortisol suppression for clinically relevant psychosocial problems during dexamethasone was only 50%. In addition, the positive predictive value of severe cortisol suppression for clinically relevant sleeping problems during dexamethasone was 30%.

Dexamethasone levels as predictor of side effects

Serum dexamethasone trough levels after 4 days of dexamethasone were available for 24 patients (50%), and showed a median of 3.97 ug/L (IQR: 1.45, 11.81). Dexamethasone trough levels were not associated with dexamethasone-induced neuropsychological problems, based on the SDQ-Dut for psychosocial problems and the SDSC for sleeping disorders.

DISCUSSION

This is the first study that investigated predictors of dexamethasone-induced neuropsychological side effects in pediatric ALL patients. The very low dose DST did not accurately predict neuropsychological problems of dexamethasone treatment. In addition, dexamethasone trough levels did not influence neuropsychological side effects.

Interestingly, however, patients being most sensitive to dexamethasone-induced suppression of the HPA-axis, experienced significantly more behavioral and sleeping problems including excessive somnolence during dexamethasone treatment. These results suggest, that children with relative glucocorticoid hypersensitivity are at risk for depressive symptoms during dexamethasone courses, as these specific behavioral problems have been identified as possible symptom of childhood depression. It has to be acknowledged, that the clinical presentation of depression in children differs from adults, in particular the occurrence of behavioral problems and less anhedonia. Because of these differences between children and adults, recently, the diagnosis of 'Disruptive Mood Dysregulation Disorder' was added to the Diagnostic and Statistical Manual of Mental Disorders 5th edition. This disorder is characterized by severe and recurrent temper outbursts with irritable or angry mood in between.[19] Interestingly, these types of behavioral problems overlap with the ones that have been frequently reported in pediatric ALL patients on dexamethasone.[20, 21]

The mechanism behind the increased neuropsychological side effects in the patients with hypersensitive responses is unknown. We could not find evidence that this was mediated by differences in pharmacokinetics, as dexamethasone levels were not associated with the extent of cortisol suppression. However, the influence of variable dexamethasone pharmacokinetics on neuropsychological side effects should be analyzed more extensively in a larger cohort by including the peak levels and the area under the curve.

Although the DST is not suitable as diagnostic tool for neuropsychological side effects, it may give us more insight in the pathophysiologic mechanism behind these side effects. Up until now it is not clear what determines the inter-individual differences in glucocorticoid sensitivity in response to the very low dose DST. There might be a role for genetic variation. Glucocorticoid receptor (GR) polymorphisms are associated with both glucocorticoid sensitivity and depression.[22] For example, harboring the ER22/23EK polymorphism (rs6189 and rs6190, G>A, located on exon 2) seems to protect against the development of adrenal insufficiency after high-dose glucocorticoid therapy[23], and seems to be relatively protective for cognitive effects of increased HPA axis activity.[24] Homozygous carriers of the BclI polymorphism (rs41423247, C>G, located 646 nucleotides downstream from exon 2) have an increased susceptibility for prolonged adrenal insufficiency[23], and have an increased risk for a major depression.[24] We did not analyze

genetic variants in our study because large numbers of participants would be needed to power the study.

The very low dose DST, which is an easy test with a low burden for patients, may be used during maintenance phase to identify patients with prolonged adrenal insufficiency. One third of our study population had a suppressed morning cortisol in a week of maintenance phase preceding a dexamethasone course. These children are at risk for life-threatening complications and may benefit from glucocorticoid replacement therapy during periods of serious stress in the first weeks after cessation of dexamethasone.[25] Adrenal insufficiency after glucocorticoid therapy in pediatric ALL patients has been previously reported.[25, 26] The impaired stress response and an inadequate host defense against infections in these patients, remains a cause of morbidity and death in childhood.[27] Hypoglycemia after prolonged fasting, which can be a sign of adrenal insufficiency, has been reported as a common adverse effect of maintenance therapy[28], and could potentially affect behavior, mood and sleep. However, fasting glucose levels during dexamethasone were not associated with neuropsychological side effects nor with the magnitude of HPA-axis suppression in our study population.

In conclusion, the very low dose DST was not an appropriate diagnostic tool to predict dexamethasone-induced neuropsychological problems in pediatric ALL patients. In addition, dexamethasone trough levels did not influence neuropsychological side effects. The association with a hypersensitive response in the very low dose DST and behavioral and sleeping problems, however, points to a possible role of genetic variation in the occurrence of neuropsychological side effects that vary widely between patients. Therefore, future studies on genetic variation are needed to better understand the pathophysiology of these toxicities, which is important for developing interventions to improve the quality of life during the maintenance phase.[5]

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Chapter 7

Eating behavior during dexamethasone treatment in children with acute lymphoblastic leukemia.

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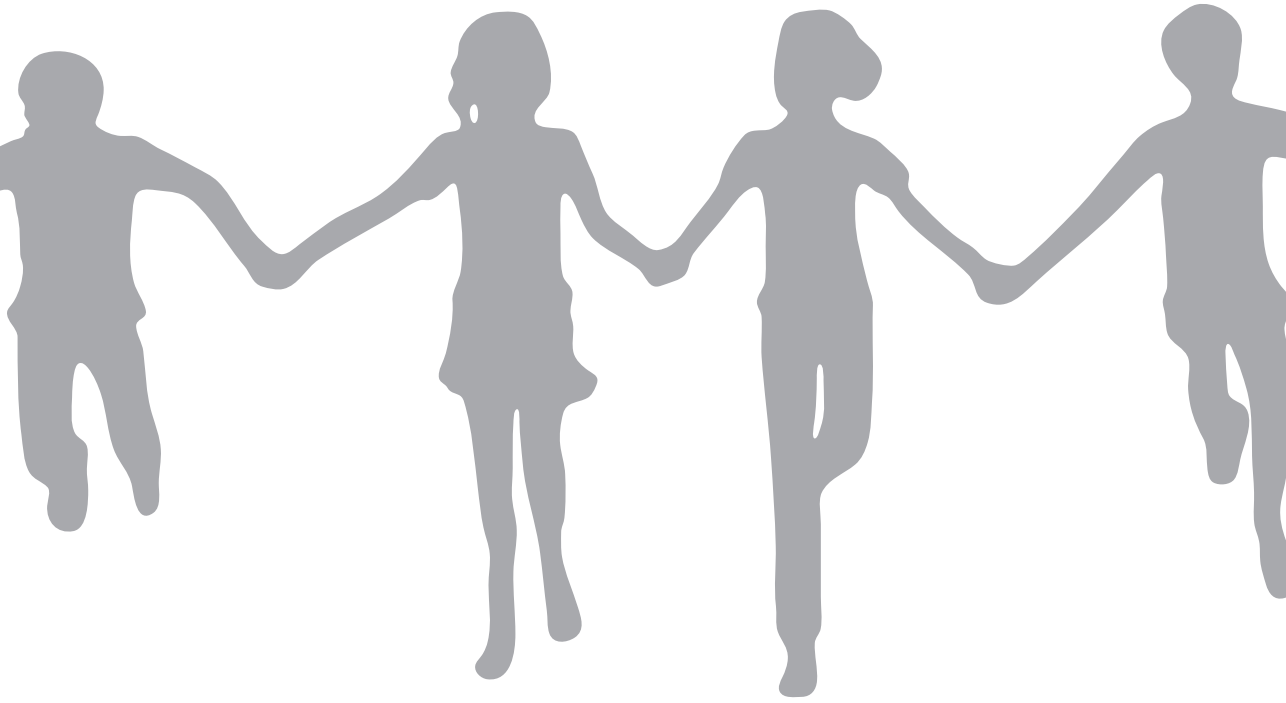

Submitted.

ABSTRACT

Background: Large prospective studies on dexamethasone-induced changes in eating behavior, energy and nutrient intake are lacking in pediatric acute lymphoblastic leukemia (ALL).

Objectives: We prospectively studied eating behavior, energy and nutrient intake during dexamethasone administration in children with ALL.

Methods: Parents of ALL patients (3-16 years) completed a dietary diary for their child during 4 days of dexamethasone (6mg/m^2) administration. Energy intake and nutrient intake (energy percentiles=E%) were assessed and compared with the recommended intake. The Dutch Eating Behavior Questionnaire for Children was completed before start and after 4 days of dexamethasone by patients 7-12 years of age.

Results: Energy intake per day (kcal) (N=44) increased significantly during dexamethasone *(*median day 1: 1103 (717-1572) versus day 4: 1482 (1176-1822), $P<0.01$), including an increase of total protein, fat, saturated fat, carbohydrate, and sodium intake. Intake of saturated fat (median day 4: 12E%) and salt (median day 4: 1.9 gram/day) exceeded the healthy range for age and gender. With respect to eating behavior, dexamethasone significantly decreased restrained eating ($P=0.04$).

Conclusions: Four days of dexamethasone treatment significantly increased energy intake, including excessive saturated fat and salt intake, and changed eating behavior in children with ALL. Nutritional and behavioral interventions during dexamethasone treatment are recommended to stimulate a healthy lifestyle.

INTRODUCTION

Dexamethasone is an important component of the treatment of pediatric acute lymphoblastic leukemia (ALL), but it is notorious for its induction of various serious side effects. One of the frequently reported side effects in ALL patients is increased appetite.[1] Even short-term glucocorticoid treatment can lead to an altered eating behavior and increased food intake.[2, 3] Still, prospective studies addressing this issue are scarce and include a limited number of patients.[1, 2]

The effect of glucocorticoids on appetite is not completely unraveled, but seems to be regulated through changes in gene expression of the hypothalamic appetite regulatory peptides, for example neuropeptide Y.[4] In the acute situation, for example by a stressor, cortisol inhibits food intake[5, 6] and liberates energy from resources to meet the energetic demands for fight or flight. In contrast, chronic glucocorticoid administration stimulates feeding.[7, 8]

Animal studies have reported that glucocorticoids increase salt appetite by increase of water and sodium excretion[9], but the changes in food preference of ALL patients during dexamethasone treatment have not been studied. Dexamethasone-induced psychosocial stress[10] could contribute to unhealthy food preferences in ALL patients.[11] Psychosocial stress has been previously associated with food responsiveness, which reflects eating in response to external food-related cues like the sight, smell, and taste of food, regardless of their physical need for food.[12]

Repetitive five-day dexamethasone courses are administered for at least 1,5 years during maintenance therapy of the most recent and ongoing Dutch Childhood Oncology Group (DCOG) ALL protocols. The suggested concomitant increased energy intake during dexamethasone courses together with impaired physical activity[1, 2] and other dexamethasone-induced metabolic toxicities[13-16] may contribute to the higher risk of developing obesity and metabolic syndrome in ALL survivors with consequent cardiovascular sequelae.[17, 18] A recent study in 784 American ALL survivors showed that 34% has metabolic syndrome.[19]

To date, neither dexamethasone-induced changes in nutrient or energy intake have been studied in a large cohort of ALL patients in the period of dexamethasone-pulses. Therefore, we investigated this in a prospective study. Secondly, we describe the underlying emotional, external and restraint eating behavior, leading to the excessive dietary pattern during dexamethasone treatment.

METHODS

Patients

Children with ALL, aged 3 to 16 years, receiving oral dexamethasone (6 mg/m^2/day) in the maintenance phase of Dutch Childhood Oncology Group ALL protocols were included. In this phase of treatment dexamethasone was administered together with vincristine 2 mg/m^2 on day 1, 6-mercaptopurine 50 mg/m^2 daily and methotrexate 30 mg/m^2 weekly. The part of the maintenance phase in which the study was performed, contained 19 five-day dexamethasone courses. The children were studied prospectively during one of these dexamethasone courses. The median start of the study was in the fourth dexamethasone course after stop of asparaginase. During the first four days of this dexamethasone course, energy and nutrient intake were registered. Eating behavior was measured at baseline before start of dexamethasone (T1) and at day 5 of treatment (T2). Weight (kg) and height (cm) were measured at T1 to calculate body mass index (BMI). Cut-off values for BMI corrected for age and sex were obtained from Cole *et al.*[20]

Energy and nutrient intake

Energy intake was calculated over four consecutive days during the dexamethasone pulse, from parent-reported dietary diaries. Parent-reported diaries during four days of dexamethasone treatment were complete for 44 patients. Food intake data were converted to energy intake (kcal) using the database of the chemical composition of foods in the Netherlands[21]. Macronutrients and sodium intake was assessed from the dietary diaries by a dietician. Data on the recommended daily allowance of macronutrients (protein: ≤25 energy percentiles (E%), carbohydrate: 40-70 E%, total fat: ≤40 E%, saturated fat: ≤10 E%) and sodium (3 years: 1.0 g/day, 4-8 years: 1.2 g/day, 8-16 years: 1.5 g/day) were retrieved from the Health Council of the Netherlands, and were corrected for age and gender.[22] The resting metabolic rate was calculated with use of the Schofield equation with correction for age and gender.[23] To calculate the individual energy requirements based on Schofield we used the following formula: energy needs = (resting metabolic rate x (activity factor (1.1)+ disease factor (1.2) -1) x growth factor (1)) / energy absorption coefficient (0.98).[24]

Eating behavior

Data on eating behavior were collected at baseline before start of dexamethasone (T1), and at day 5 (T2) of the dexamethasone course. Eating behavior was measured by the Dutch Eating Behavior Questionnaire for Children (DEBQ-C: 7-12 years)[25]. The DEBQ-C has three subscales: Restrained Eating (7 items), Emotional Eating (7 items), and External Eating (6 items); a higher score on each subscale reflects the more pronounced presence of problems. The Restrained Eating scale assesses both intentions to restrict food intake

and actual behavioral restrained (dieting). Emotional eating means eating in response to emotional arousal states such as fear, anger of anxiety. External eating means eating in response to external food cues such as sight and smell of food. The DEBQ-C has been validated for use in children 7-12 years of age and was therefore completed only by all 17 patients 7-12 years of age.

Statistics

The recommended daily allowance and the intake were compared with a Wilcoxon Signed Rank test. Spearman's coefficient described the correlation r between energy and nutrient intake and gender, age and baseline BMI. The effect of dexamethasone on eating behavior was assessed comparing the results at T1 and T2 by a Wilcoxon Signed Rank test. The differences in eating behavior for different categories, based on DEBQ-C subscores, were compared with a Mann-Whitney U test. All analyses were performed using SPSS, version 21.

RESULTS

Baseline characteristics

Fifty patients were included (46% males). Their median age was 6.0 years (interquartile range (IQR): 4.0, 10.0). The median week of maintenance phase was week 37 (IQR: 31, 48). The median weight SDS was 0.13 (IQR: -0.48, 0.86). At T1, based on BMI[20], 19% was overweight, and obesity was present in 4% of the patients. In the general population of the Netherlands the prevalence of overweight and obesity in this age category are 14% and 2% respectively.[26]

Energy intake

The dietary diaries were completed for 44 children. At day 1 the median energy intake was 19% lower than the recommended energy intake. The median energy intake per day increased significantly during four days of dexamethasone (median day 1: 1103 (IQR: 717, 1572) versus median day 4: 1482 (IQR: 1176, 1822), $P<0.01$) (Figure 1), resulting in a median energy intake at day 4 that was 9% higher than the recommend energy intake (median: 1306 (IQR: 1139, 1707). The number of patients with an energy intake above their individual energy requirements increased from day 1 to day 4 (11 patients (25%) to 28 patients (64%) respectively). Increased energy intake at day 4 was associated with younger age (r=-0.41, $P<0.01$). The energy intake (corrected for age and gender) was independent of the week of maintenance phase, in which the patient participated.

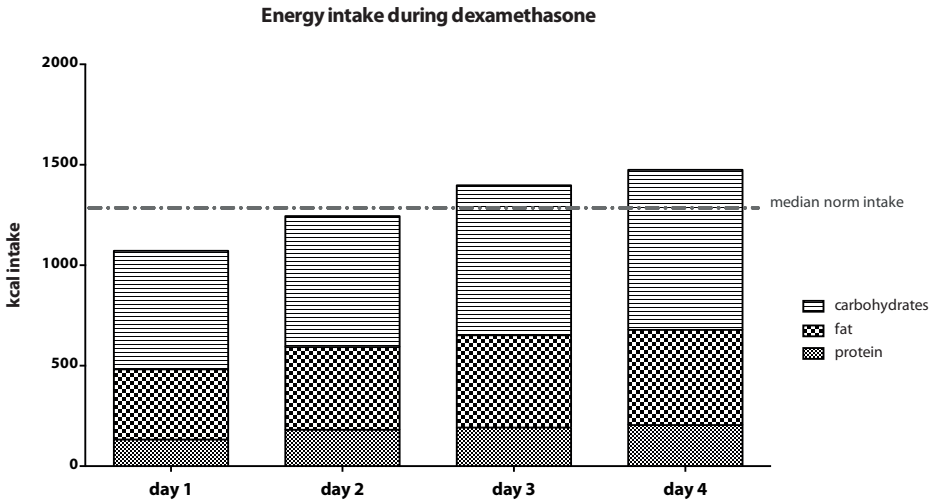

Figure 1. *Median total energy intake (kcal) per treatment day, divided in the median contributing percentiles proteins, fat, and carbohydrates during four days of dexamethasone. The median norm intake (based on the formula: energy needs = (resting metabolic rate x (activity factor (1.1)+ diseas e factor (1.2) -1) x growth factor (1)) / energy absorption coefficient (0.98)[24]) is depicted with the dashed grey line.*

Macronutrient and sodium intake

The protein intake (median day 1: 1.3 g/kg (IQR: 0.9, 2.3) versus day 4: 2.1 g/kg (IQR: 1.5, 2.8), *P*<0.01), total fat intake (median day 1: 1.5 g/kg (IQR: 0.8, 2.4) versus day 4: 2.4 g/kg (IQR: 1.3, 3.0), *P*<0.01), saturated fat intake (median day 1: 0.6 g/kg (IQR: 0.3, 1.0) versus day 4: 1.0 g/kg (IQR: 0.5, 1.3), *P*<0.01), and carbohydrate intake (median day 1: 4.9 g/kg (IQR: 3.5, 8.8) versus day 4: 8.9 g/kg (IQR: 5.2, 11.9), *P*<0.01) increased significantly during the dexamethasone course. (Figure 1) In addition sodium intake increased significantly during the course (median day 1: 1.4 g/day (IQR: 1.0, 2.0) versus day 4: 1.9 g/day (IQR: 1.2, 2.9), *P*<0.01). (Figure 2)

The energy percentages of protein, fat, and carbohydrate contributing to daily intake (E%), did not significantly change. (Figure 1) The median E% of protein (14%), fat (32%), and carbohydrate (54%) intake were within normal limits of the recommended daily allowance. In contrast, the median E% of saturated fat (median day 1 and day 4: 12 E%) was constantly higher than recommended, and medium sodium intake exceeded the healthy limit during the dexamethasone pulse (median day 4: 1.9 g/day). Nutrient intake (E%) was independent of age and gender.

Eating Behavior

The DEBQ was completed for 17 children in the age of 7-12 years. Before start of dexamethasone Emotional Eating and Restrained Eating were decreased in ALL patients compared to the norm of the Dutch population (Z-score <0.00). Patients showed more

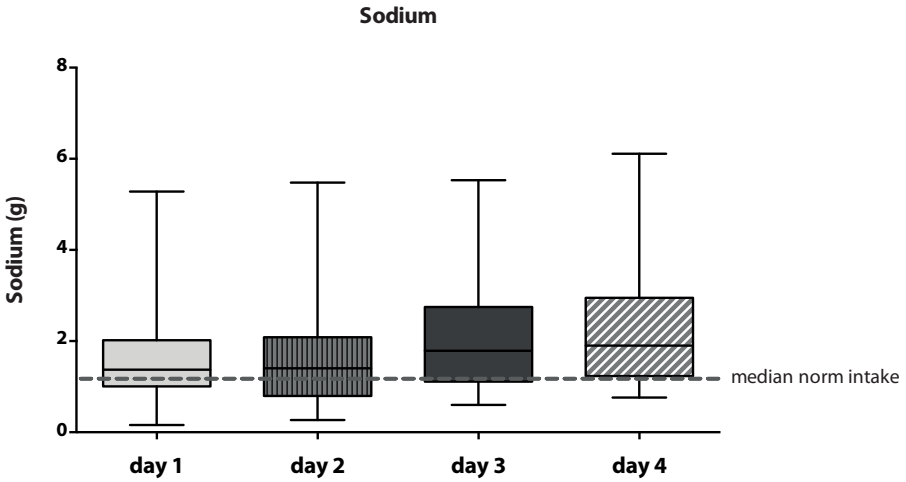

Figure 2. *Sodium intake during during four days of dexamethasone treatment. The median norm sodium intake is depicted with the dashed line.*

External Eating at baseline compared to healthy peers. Four days of dexamethasone treatment significantly decreased Restrained Eating (median: -0.43 (IQR: -0.82, 0.95) vs -0.62 (IQR: -1.06, -0.16), *P*=0.04), but did not change Emotional Eating (median: -0.74 (IQR: -0.74, 0.05) vs -0.74 (IQR: -0.74, 0.70), *P*=0.24), and External Eating (median: 0.60 (IQR: -0.24, 1.34) vs 0.59 (IQR: -0.04, 1.14), *P*=0.25) in the 17 patients. (Figure 3)

Figure 3. *Eating behavior based on Dutch Eating Behavior Questionnaire (DEBQ) on day 1 and day 5 of dexamethasone treatment. Subscores: emotional eating, external eating and restraint eating.*

As expected, patients with an increase on the subscale of Emotional Eating >0.5 (Z-score) during dexamethasone treatment had a higher total energy intake in four days than patients with less increase of Emotional Eating respectively (median: 6866 kcal (IQR: 6020, 9115) versus 5106 kcal (IQR: 4063, 6198), $P<0.04$) and a higher fat intake in four days (median: 9.3 g/kg.d, IQR: 6.2, 11.1 versus median: 5.7 g/kg.d, IQR: 3.6, 7.9, $P=0.04$).

DISCUSSION

Energy intake increased significantly during the four consecutive days of dexamethasone treatment, along significantly increased total protein, fat, saturated fat, carbohydrate, and sodium intake. Interestingly, whereas the relative contribution of protein, fat, and carbohydrate to energy intake remained within normal limits, intake of saturated fat and salt exceeded the healthy range during dexamethasone treatment. With respect to eating behavior, dexamethasone significantly decreased restrained eating.

The preferences for salty and fatty foods seem to go along, since the total excess sodium intake was associated with the total saturated fat intake during the four days of dexamethasone. Since most of the Dutch pediatric ALL patients receive dexamethasone courses for at least 1.5 years, a dexamethasone-induced high fat and salty diet have to be watched for as they may contribute to the high incidence of metabolic syndrome and consequent cardiovascular sequelae in ALL survivors.[18] Nowadays, it is unknown whether there is a causal relationship between the unhealthy diet during dexamethasone pulses and metabolic syndrome in ALL survivors. A recent study in the USA, showed that one third of ALL survivors had the metabolic syndrome.[19] Increased fat consumption and an overall unhealthy diet, which is also reported in long-term ALL survivors[27], has already shown to be associated with the occurrence of metabolic syndrome.[28] For example, high salt intake could lead to hypertension, but is also a potential risk factor for obesity.[29] The higher incidence of adiposity and obesity in the study population can also contribute to the increased risk of metabolic syndrome, since Weiss *et al.* reported that the prevalence of metabolic syndrome among obese children is high and increases with worsening obesity.[30]

With respect to eating behavior, our results are limited to the children aged 7 to 12 years. Patients under the age of 7, however, also had unhealthy diets during dexamethasone treatment, and a higher energy intake than recommended was associated with younger age. It would therefore be very interesting to study their emotional and external eating behavior, which may be higher due to less developed impulse control in this age group. Future studies should include these young children.

Although the long-term effects of high saturated fat and salt intake, together with the increased energy intake and extreme eating behavior during dexamethasone pulses need to be studied more extensively, the unhealthy eating pattern in children treated for ALL with dexamethasone, emphasizes the need for nutritional and behavioral interventions. Behavioral problems, accompanying dexamethasone treatment[10] could hinder nutritional interventions, underscoring the need for developmental- and age-directed coaching by professionals, closely involving the parents. Early intervention may prevent an unhealthy lifestyle post-treatment, which could increase the risk for obesity, metabolic syndrome, and consequent cardiovascular sequelae later in life.

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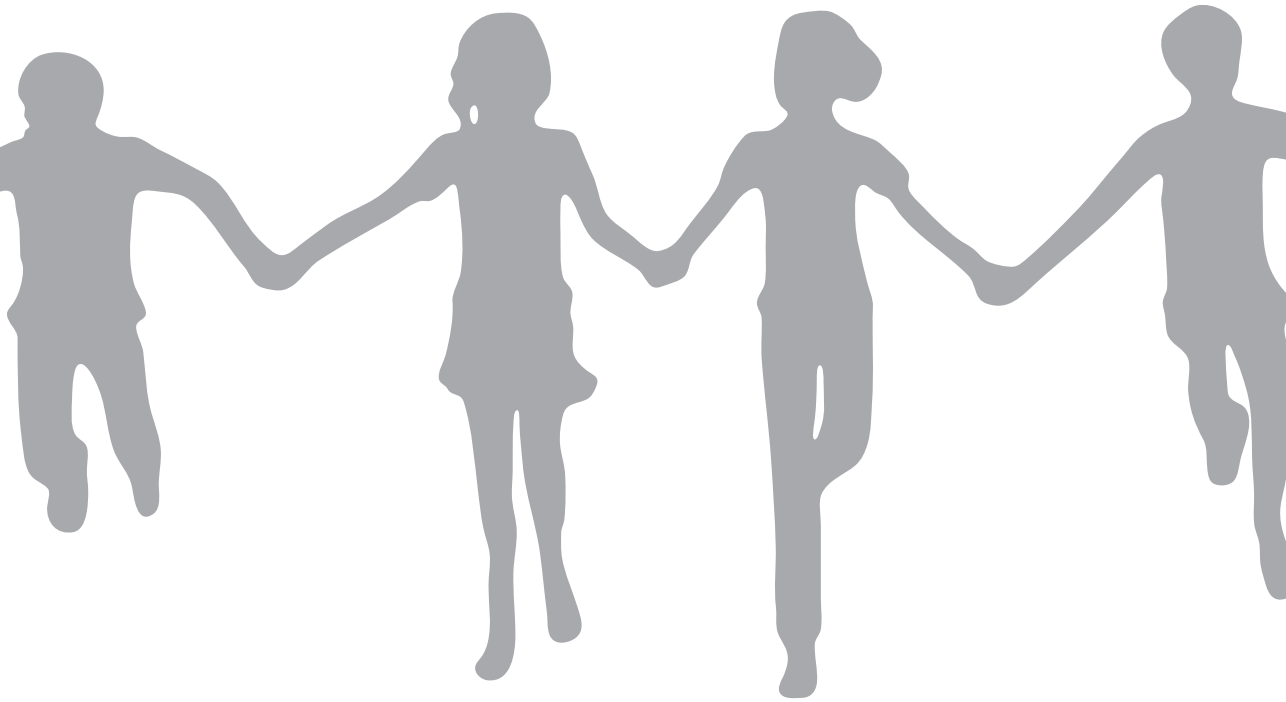
Chapter 8

General discussion and future perspectives



Hydrocortisone as novel intervention for neuropsychological side effects

The results of this thesis suggest that hydrocortisone addition provides a valuable intervention for clinically relevant dexamethasone-induced psychosocial and sleeping problems in children with acute lymphoblastic leukemia (ALL). It is the first randomized controlled clinical trial that investigated whether an intervention with a low patient burden can be used to reduce dexamethasone-induced side effects. The beneficial effects for a subset of 38% of patients are promising, since current therapeutic options for dexamethasone-induced neuropsychological side effects are scarce, and limited to antidepressants and antipsychotics with accompanying side effects.[1-4]

Before introducing this novel intervention to clinical practice, however, the beneficial effects need validation in larger cohorts of patients with clinically relevant neuropsychological side effects. Primarily, because a quarter of the participating patients in our study did not experience neuropsychological side effects, which could have influenced the results of the total study population. Regression to the mean may have influenced results.

Till validation, pediatric oncologists may incidentally consider the use of hydrocortisone for ALL patients with extreme dexamethasone-induced behavioral, depressive or psychotic symptoms, and start hydrocortisone administration in a circadian rhythm during dexamethasone treatment. In such patients the severity of the side effects outweigh the lack of validation, especially because physiological dosages of hydrocortisone have a low patient burden and has proven to be safe at the short-term. Further research is needed for long-term safety, although theoretically no issues are foreseen.

Prediction of patients that may benefit from this novel intervention

Before implementation of hydrocortisone as intervention for neuropsychological side effects in clinical practice, it may be important to select those that will benefit from hydrocortisone addition. Upfront we hypothesized that these side effects were influenced by glucocorticoid sensitivity at the tissue level and may therefore be predicted by a very low-dose dexamethasone suppression test. Our results, however, showed that, although a correlation was found between glucocorticoid hypersensitivity and more behavioral and sleeping problems during dexamethasone, this test is not an adequate tool to select individual patients. *(Chapter 6)*

Since prediction tools are lacking, patients with clinically relevant neuropsychological side effects need to be selected during dexamethasone treatment. The parent-reported Strengths and Difficulties Questionnaire (SDQ) and the parent-reported Sleep Disturbance Scale for Children (SDSC) have already shown to be valuable tools to quantify psychosocial problems and sleeping problems during dexamethasone treatment. *(Chapter 4)* These parent-reported questionnaires are validated for patients 3-16 years of age

and are not time-consuming and convenient in a clinical setting, in contrast to other behavioral questionnaires, like the Child Behavior Checklist[5] and the Behavior Rating Inventory of Executive Function[6]. The Behavior Rating Inventory of Executive Function has a parent-reported short version with 24 items[7], but is validated for children from 5 years of age.

Based on recent changes in the Diagnostic and Statistical Manual of Mental Disorders, behavioral problems in children could be a symptom of depression, which has a different clinical presentation in childhood, compared to depression in adults. Since behavioral problems are frequently seen during dexamethasone treatment, a questionnaire focusing on depressive symptoms, for example the 13-item Short Mood and Feelings Questionnaire could also be valuable to select patients with neuropsychological problems. This brief instrument evaluates depressive symptoms of children aged 8-18 years, and may be a short and practical option in a follow-up study.[8]

A limitation of our study was the use of the self-reported Dutch Eating Behavior Questionnaire, which was only validated for children aged 7-12 years.[9] As a consequence data on eating behavior in younger children are missing. An increased energy intake during dexamethasone, however, was associated with younger age, so a parent-reported questionnaire on eating behavior, like the Child Eating Behavior Questionnaire[10], for these younger children would be valuable.

Since we aim to improve quality of life by diminishing neuropsychological side effects, questionnaires assessing the quality of life like the 23-item Pediatric Quality of Life Inventory 4.0[11] could be valuable too. This questionnaire takes only five minutes to be completed and has a parent- and child-reported version.[11]

Potential improvements of the intervention

Hydrocortisone was administered in our patients in order to restore the balance between the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR) activity in the brain that become under activated and over activated respectively by dexamethasone treatment.[12-14] Besides, the circadian rhythm of administration is essential to mimic the natural daily pattern of cortisol secretion[12], which was emphasized by different studies. In patients with adrenal insufficiency, who lack cortisol secretion[15], cortisol replacement without mimicking the physiological circadian rhythm leads to more side effects, like hyperglycemia and insulin resistance, by overexposure during parts of the day when cortisol exposure is normally low[16]. Low nighttime cortisol levels in patients with Addison's disease might also lead to sleep disturbances.[17] Normal nighttime cortisol levels in healthy humans are necessary for normal rapid eye movement sleep regulation.[18] Although our treatment schedule replenishes cortisol in a three times daily dosing schedule, the normal biorhythm of cortisol is not completely mimicked. Based on a study of Mah *et al.*[19], this treatment renders some patients overtreated immediately after oral ad-

ministration and undertreated within a few hours. During nighttime and early morning, the glucocorticoid levels during oral hydrocortisone treatment are undetectable, which is in contrast with the rise seen in healthy individuals[20]. This may be an explanation for the fact that not all neuropsychological side effects completely disappeared in our study patients. Recently, continuous subcutaneous infusion of hydrocortisone in Addison's disease successfully mimicked the physiological cortisol rhythm.[21] Although this might be a treatment option in ALL patients who poorly respond to oral hydrocortisone, it has a higher patient burden and it is not practical during the maintenance phase, in which patients visit the outpatient clinic only once a week.

Our current treatment schedule with hydrocortisone administration should remain limited to the period in which dexamethasone courses are administered. Glucocorticoid administration during off-dexamethasone weeks could lead to adrenal insufficiency[22], an impaired stress response, and an inadequate host defense against infections[23], although this is only expected when using supra-physiological doses.

Future research may focus on developing more selective glucocorticoids to prevent unwanted side effects. As an example, recently, a selective GR modulator, C108297, was developed with suppressive effects of stress-induced hypothalamus-pituitary-adrenals (HPA) axis activity in the brain in rats, but without unwanted stimulatory effects on amygdala CRF that would affect systemic basal cortisol levels.[24] The development of selective glucocorticoids that have anti-leukemic effects but lack side effects, would overcome the burden of the dexamethasone-induced neuropsychological toxicities.

Interventions for acute metabolic side effects and changes in eating behavior

Beside its neuropsychological side effects, dexamethasone acutely influenced components of the metabolic syndrome, and induced an increased energy intake in more than 60% of patients. Hydrocortisone had no beneficial effect on these side effects, presumably because the pathophysiologic mechanisms of various side effects differ.[25-27] For example, dexamethasone-induced hypertension may be due to inotropic and vasoconstrictive effects of glucocorticoids on the cardiovascular system[25], while hyperglycemia is induced by inhibition of glucose uptake in peripheral tissues and stimulation of hepatic gluconeogenesis.[25] Dexamethasone-induced weight gain is influenced by changes in eating behavior[28], changes in physical activity[29], and GR-dependent stimulation of adipocyte hypertrophy[30] especially in visceral adipose tissue[31]. We found that high dexamethasone trough levels were associated with high glucose levels *(Chapter 5*), which emphasizes the need for more extensive pharmacokinetic studies that include measurement of peak levels and area under the curve, in a large cohort of ALL patients.

The acute effects of dexamethasone on components of the metabolic syndrome (hyperglycemia, hypertriglyceridemia, and hypertension), the increased energy intake[32, 33], and the unhealthy eating pattern (especially more saturated fat and salt), could contrib-

ute multifactorially to the high incidence of metabolic syndrome in childhood leukemia survivors.[34, 35] Therefore, interventions should not only focus on short-term toxicities, but also on the long-term risk of diabetes and cardiovascular diseases.[35] Such interventions could include nutritional and behavioral interventions during maintenance treatment, to stimulate a healthy lifestyle. Physical activity, which is decreased during dexamethasone treatment in children with ALL[29], should be encouraged. It may, however, be hard for patients to adhere to exercise programs during two years of ALL treatment.[36] The inter-patient variability in eating behavior during dexamethasone suggests that, interventions may be most effective if designed in a patient-tailored way. Behavioral problems, accompanying dexamethasone treatment could hinder nutritional interventions, underscoring the need of developmental- and age-directed coaching by professionals. Parents should be closely involved in these interventions.

Variability in neuropsychological side effects

Dexamethasone-induced neuropsychological side effects vary widely between patients. 38% of the study population developed clinically relevant psychosocial and sleeping problems. Future studies should explore whether genetic variation plays a role in this wide variety. Studies in other patient groups have found gene variants in the MR and GR that may contribute to inter-individual differences. For example, MR haplotype 2 is associated with dispositional optimism and protects against depression.[37] Also, GR polymorphisms have been shown to be associated with both glucocorticoid sensitivity and depression.[38] For example, harboring the ER22/23EK polymorphism seems to protect against the development of adrenal insufficiency after high-dose glucocorticoid therapy[39], and seems to be relatively protective for cognitive effects of increased HPA axis activity.[40] Carriers of a homozygous Bcl1 polymorphism have an increased susceptibility for prolonged adrenal insufficiency[39], and have an increased risk for a major depression.[40]

Differences in sensitivity of the HPA-axis feedback to glucocorticoids may also contribute to the inter-patient variability in neuropsychological side effects. This thesis showed that patients with a hypersensitive HPA-axis feedback experienced more behavioral problems, total sleeping problems, and excessive somnolence during dexamethasone treatment. (*Chapter 6)* The mechanism behind the increased problems in patients with hypersensitive responses is unknown.

Patients with HPA-axis hypersensitivity showed some overlap in occurrence of clinically relevant neuropsychological side effects. However, a large group of patients with clinically relevant side effects did not have a hypersensitive response in the very low-dose DST (see Figure 1). Patients with glucocorticoid hypersensitivity may be more prone to develop MR:GR imbalance.

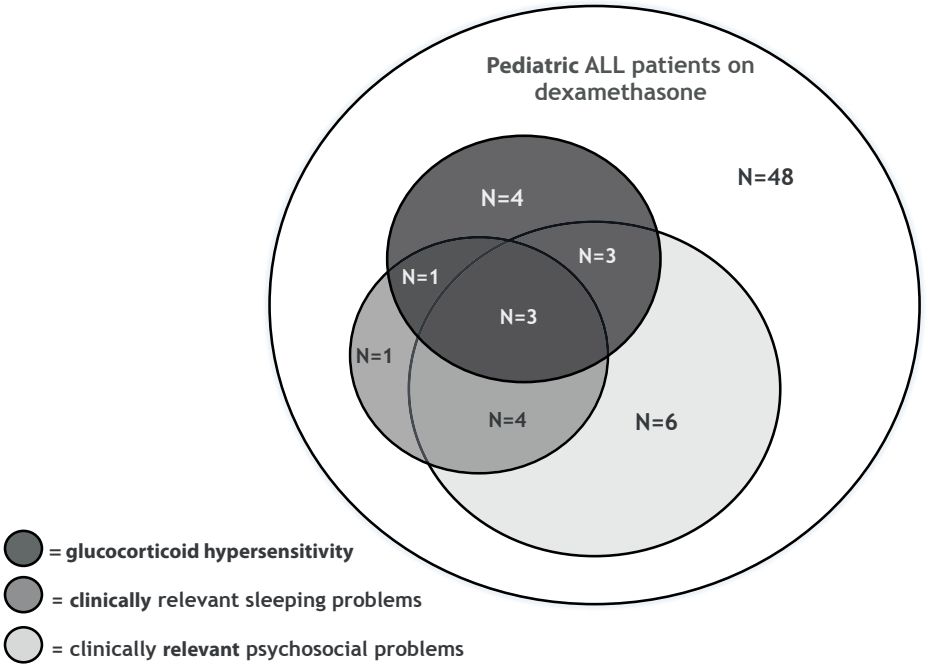


Figure 1. *Overlap of patients with clinically relevant psychosocial side effects (SDQ), patients with clinically relevant sleeping problems (SDSC), and patients with HPA-axis hypersensitivity.*

Future studies should focus on whether genetic variances that are known to play a role in depression and/or glucocorticoid sensitivity influence neuropsychological side effects in children with ALL. Genetic profiling is unlikely to become an individual diagnostic tool for neuropsychological side effects, but it may give further insight in the pathophysiology.

Pharmacokinetics did not seem to influence neuropsychological side effects. This was expected based on our hypothesis, as every patient will have a suppressed endogenous cortisol production during treatment with 6mg/m^2 dexamethasone. Since we only measured trough levels, however, future pharmacokinetic studies need to investigate the influence of dexamethasone levels on neuropsychological side effects more extensively while including peak levels and area under the curve, and by analyzing a larger cohort. Children with higher dexamethasone levels, due to lower clearance, may for example become more hypersensitive to glucocorticoids.

Applicability of intervention for other patients

The current Dutch medium risk ALL treatment protocol includes 5-day dexamethasone courses of 6 mg/m^2 in cycles of 3 weeks during at least one and a half year. Patients

with rheumatic diseases or idiopathic thrombocytic purpura receive lower doses of dexamethasone. Neuropsychological side effects, however, have also been described during prednisone treatment in children with ALL.[4, 41-43] These side effects do not seem to differ between dexamethasone and prednisone based on randomized controlled trials, but a prospective study of Pound *et al.*[44] reported more behavioral side effects with dexamethasone. (*Chapter 2*) Based on our hypothesis the MR:GR balance would be less severely disturbed during prednisone treatment, because prednisolone, in contrast to dexamethasone, has some affinity for the MR beside its affinity for the GR. Prednisolone will bind the GR and MR with a ratio of 5:1, so there is still some MR activation.[45] Prednisone treatment, however, still leads to overactivation of the GR and suppression of circadian rhythm of the endogenous cortisol production, so patients receiving prednisone may also benefit from hydrocortisone. This hypothesis is supported by a case report of a patient with Cushing's syndrome who needed synthetic glucocorticoid treatment after bilateral adrenalectomy, and developed severe psychotic symptoms that were unresponsive to psychotropic drugs as long as she was taking prednisone as replacement therapy. After she was switched to a regimen including cortisol that leads to more occupation of the MR, the psychopathology disappeared.[46]

The dexamethasone pulses during maintenance therapy in ALL are currently under discussion, but even if these pulses would be removed from maintenance therapy in future protocols, the novel intervention with hydrocortisone could still be valuable during other phases of dexamethasone treatment in the ALL protocols.

In conclusion, pediatric ALL patients with clinically relevant neuropsychological problems during dexamethasone treatment seem to benefit from a physiological dose of hydrocortisone in a circadian rhythm. This novel therapeutic option, however, needs to be validated before introducing it into routine clinical practice. Apart from the SDQ and SDSC, short parent-reported depression-, eating behavior-, and quality of life questionnaires could be valuable for selection of patients that may benefit from this intervention. This selection is needed because not all patients experience clinically relevant neuropsychological problems. Future pharmacogenomic and pharmacokinetic studies may give more insight in the variation in these side effects between patients.

Apart from neuropsychological problems, dexamethasone treatment can lead to acute metabolic toxicity with a higher risk of the development of components of the metabolic syndrome. Therefore, patient-tailored nutritional and behavioral interventions are needed to improve a healthy lifestyle during maintenance treatment.

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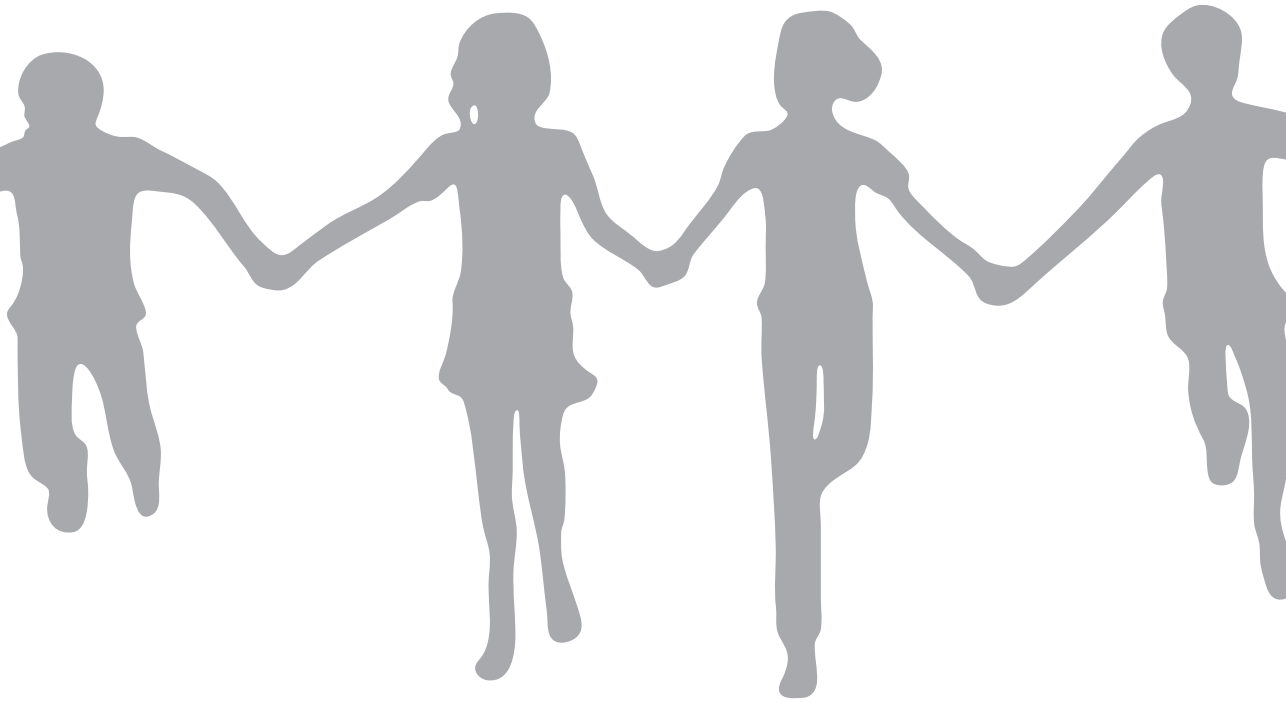
Chapter 9

Summary & Samenvatting



SUMMARY

Synthetic glucocorticoids, such as dexamethasone and prednisolone, are widely and successfully used as anti-leukemic agents[1, 2], like in the Dutch treatment protocols for pediatric acute lymphoblastic leukemia (ALL). Dexamethasone is considered to be the preferred glucocorticoid based on its higher anti-leukemic activity[3] and central nervous system penetration.[1] However, the use of glucocorticoids, and especially dexamethasone, is notorious for various side effects, like metabolic and neuropsychological side effects.[4-7] Neuropsychological side effects are experienced as the most detrimental to the quality of life by patients and their caregivers.[8] Since most pediatric ALL patients receive dexamethasone courses during at least one and a half year, these side effects have a high impact on the daily life and development. To date, therapeutic interventions to avoid dexamethasone-induced neuropsychological side effects are scarce, and limited to anti-depressive agents and antipsychotics.[9-11] Therefore, there is a strong need for novel interventions to reduce these side effects. In this thesis we studied the efficacy of hydrocortisone as intervention for dexamethasone-induced neuropsychological side effects, based on the hypothesis that the neuropsychological side effects are due to cortisol depletion of the mineralocorticoid receptor (MR) caused by dexamethasone. [16, 17]

Our systematic review of literature showed no clinically meaningful differences between the effect of dexamethasone and prednisone on behavioral problems, mood disorders, cognitive effects, or sleeping problems[5, 12-15](**Chapter 2**).

We hypothesized that the neuropsychological side effects of dexamethasone in children with ALL are due to cortisol depletion of the MR caused by dexamethasone[16, 17], which can be overcome by hydrocortisone addition. Before performing a clinical study, we needed to establish that MR activation with hydrocortisone did not interfere with the anti-leukemic activity of dexamethasone. Our *in vitro* study showed that hydrocortisone did not affect glucocorticoid sensitivity of ALL cell lines nor ALL cells primarily obtained from patients with different leukemic subtypes (**Chapter 3**). We also identified a very low MR expression on ALL cells, and MR expression did not differ between glucocorticoid resistant and glucocorticoid sensitive patients' cells.

The findings of this preclinical study enabled the Dexadagen study, described in **Chapter 4**, in which we investigated whether addition of a physiological dose of hydrocortisone to dexamethasone treatment decreases the neuropsychological side effects and metabolic side effects in children with ALL. In this multicenter double blind, randomized controlled trial with a crossover design, 50 patients (3-16 years) were included during two consecutive courses of dexamethasone according to the Dutch Childhood

Oncology Group ALL protocols. The patients were randomly assigned to receive either hydrocortisone or placebo in a circadian rhythm (10 mg/m^2/day) during the first or the second dexamethasone course. In the total group of patients, hydrocortisone addition had no significant effect on mood, behavior, sleep, cognition, and metabolism. However, a more detailed analysis in the nested group of 16 patients who had clinically relevant dexamethasone-induced psychosocial side effects revealed that the addition of hydrocortisone substantially reduced total psychosocial problems, emotional symptoms, behavioral problems, and the impact of the psychosocial problems in daily life. Moreover, in nine patients with clinically relevant sleep-related difficulties, hydrocortisone reduced overall sleeping problems, including initiating and maintaining sleep. In contrast, hydrocortisone addition had no effect on metabolic parameters.

We also studied the direct effects of dexamethasone administration on all components of the metabolic syndrome (**Chapter 5**). Four days of 6mg/m^2 dexamethasone administration significantly increased median fasting serum levels of HDL, LDL, total cholesterol, triglycerides, glucose and insulin. Insulin resistance (HOMA-IR>3.4) increased substantially from 8% to 85%. Dexamethasone trough levels were positively correlated with high glucose levels after four days of dexamethasone, but not with other parameters. In addition, dexamethasone significantly increased diastolic and systolic blood pressure. Altogether, we showed that even within four days of dexamethasone ALL patients developed an increment of components of the metabolic syndrome.

Identification of patients at risk for developing neuropsychological side effects would enable individualized treatment to potentially reduce dexamethasone-induced side effects. We hypothesized that neuropsychological side effects may be influenced by glucocorticoid sensitivity at the tissue level, and determined whether we could predict these side effects by a very low dose dexamethasone suppression test (DST). We found that the subset of patients with a hypersensitive response (N=13, 26%) in the DST had more dexamethasone-induced behavioral problems, overall sleeping problems, including somnolence (**Chapter 6)**. The positive predictive value of the DST for psychosocial problems and sleeping problems, however, was only 50% and 30% respectively. Therefore we concluded, that the very low dose DST is not of value as risk predictor for neuropsychological side effects. The association of behavioral problems and sleeping problems with a hypersensitive response in the very low dose DST, however, appoints to a possible role of genetic variation in the occurrence of neuropsychological side effects that vary widely between patients.

Dexamethasone trough levels were not associated with neuropsychological side effects.

Dexamethasone can also induce significant changes in eating behavior and energy intake. We prospectively studied eating behavior, energy and nutrient intake during dexamethasone administration (**Chapter 7**). The energy intake per day was excessive during one dexamethasone course, and included an increase of energy intake, total protein, fat, saturated fat, carbohydrate, and sodium intake in more than 60% of the patients. Intake of saturated fat and salt exceeded the healthy range for age and gender. There was a high inter-patient variability in dexamethasone-induced eating behavior, but dexamethasone significantly decreased restrained eating in the total group.

In conclusion, the results of this thesis suggest that hydrocortisone addition provides a valuable intervention for clinically relevant dexamethasone-induced psychosocial and sleeping problems in children with ALL. Before introducing this novel intervention to clinical practice, however, the beneficial effects need validation in larger cohorts of patients with clinically relevant psychosocial and sleeping problems.

NEDERLANDSE SAMENVATTING

Synthetische glucocorticoïden, zoals dexamethason en prednison, worden veel gebruikt in de behandeling van leukemie[1, 2] en hebben een belangrijke plaats in het Nederlandse behandelprotocol van acute lymfatische leukemie (ALL). Dexamethason heeft de voorkeur vanwege zijn sterkere antileukemische werking[3] en omdat het beter doordringt tot het centraal zenuwstelsel.[1] Helaas kan het gebruik van deze glucocorticoïden tot verschillende bijwerkingen leiden, zoals metabole en neuropsychologische bijwerkingen.[4-7] Volgens patiënten en hun ouders hebben neuropsychologische bijwerkingen de grootste impact op de kwaliteit van leven.[8] Omdat kinderen in het huidige medium-risico ALL protocol gedurende tenminste anderhalf jaar dexamethason blokken krijgen, kunnen deze bijwerkingen een grote impact op het dagelijks leven en de ontwikkeling van het kind hebben. Op dit moment zijn er weinig behandelopties voor neuropsychologische bijwerkingen van dexamethason en zijn ze beperkt tot antidepressiva en antipsychotica.[9-11] Er is daarom behoefte aan nieuwe behandelingen om de neuropsychologische bijwerkingen te verminderen. In dit proefschrift hebben we de effectiviteit van hydrocortison bestudeerd als behandeling voor neuropsychologische bijwerkingen van dexamethason. De behandeling met hydrocortison is gebaseerd op de hypothese dat neuropsychologische bijwerkingen in kinderen met ALL verminderd kunnen worden door het tekort aan cortisol binding aan de mineralocorticoïd receptor (MR), veroorzaakt door dexamethason, op te heffen.[16, 17]

Onze systematische review van de literatuur liet geen klinisch relevante verschillen lieten zien tussen dexamethason en prednison op het gebied gedragsproblemen, stemmingswisselingen, cognitieve effecten of slaapproblemen[5, 12-15] (**Hoofdstuk 2**).

Wij veronderstelden dat neuropsychologische bijwerkingen van dexamethason worden veroorzaakt door het tekort aan cortisol binding aan de MR, die veroorzaakt wordt door dexamethason.[16, 17] Tevens namen wij aan dat dit tekort aan cortisol binding kon worden opgelost door hydrocortison toevoeging.[16, 17] Voordat dit in de kliniek getest kon worden, moesten we uitsluiten dat MR activatie met hydrocortison de antileukemische werking van dexamethason niet beïnvloedt. Onze *in vitro* studie toonde aan dat hydrocortison de glucocorticoïd gevoeligheid van ALL cellijnen en ALL patiënten cellen van verschillende leukemie subtypen niet beïnvloedt (**Hoofdstuk 3**). We vonden ook een erg lage MR expressie op leukemie cellen en de MR expressie verschilde niet tussen glucocorticoïd resistente en gevoelige patiënten cellen.

De resultaten van de preklinische studie maakten de start van de Dexadagen studie mogelijk, welke beschreven is in **Hoofdstuk 4**. Hierin onderzochten we of toevoeging

van een fysiologische dosis hydrocortison tijdens dexamethason behandeling de neuropsychologische en metabole bijwerkingen in kinderen met ALL kon verminderen. In deze nationale, dubbelblinde, gerandomiseerde, placebo-gecontroleerde studie met een cross-over opzet werden 50 patiënten (3-16 jaar) behandeld met twee blokken dexamethason volgens het Nederlandse ALL protocol. De patiënten werden willekeurig ingedeeld om hydrocortison of placebo in een circadiaans ritme (10 mg/m^2/dag) te krijgen tijdens het eerste of het tweede dexamethason blok. In de totale groep patiënten had hydrocortison geen effect op gedrag, stemming, slaap, cognitie en metabole bijwerkingen. Echter, in een meer gedetailleerde analyse in de subgroep van 16 patiënten die klinisch relevante psychosociale problemen door dexamethason ontwikkelden, bleek toevoeging van hydrocortison de psychosociale problemen, emotionele problemen, gedragsproblemen en de impact van de psychosociale problemen op het dagelijks leven, substantieel te verminderen. Daarnaast, verminderde hydrocortison in negen patiënten met klinisch relevante slaapproblemen, het totaal aantal slaapproblemen en de problemen met in slaap vallen en blijven. In tegenstelling tot het gunstige effect voor neuropsychologische problemen, had hydrocortison geen effect op metabole bijwerkingen.

We onderzochten ook de acute effecten van dexamethason op alle componenten van het metabole syndroom. (**Hoofdstuk 5**) Vier dagen behandeling met 6mg/m^2 dexamethason leidde tot een significante stijging van nuchtere spiegels van HDL, LDL, totaal cholesterol, triglyceriden, glucose en insuline. Insuline resistentie (HOMA-IR>3.4) steeg substantieel van 8% naar 85%. Dexamethason dal spiegels bleken positief gecorreleerd aan hoge glucose waarden na vier dagen dexamethason. Daarnaast leidde dexamethason tot een significante stijging van de diastolische en systolische bloeddruk. Samenvattend ontwikkelden kinderen met ALL tijdens slechts vier dagen dexamethason behandeling componenten van het metabool syndroom.

Het herkennen van patiënten die kans hebben op het ontwikkelen van neuropsychologische bijwerkingen zou geïndividualiseerde behandeling mogelijk maken met als doel dexamethason-geïnduceerde bijwerkingen te verminderen. Wij veronderstelden dat deze bijwerkingen werden beïnvloed door glucocorticoïd gevoeligheid op weefselniveau en onderzochten of we de bijwerkingen konden voorspellen met een 'zeer lage dosis' dexamethason suppressie test (DST). Wij vonden dat de subgroep van patiënten met een hypersensitieve reactie in de DST (N=13, 26%) meer dexamethason-geïnduceerde gedragsproblemen, slaapproblemen en extreme vermoeidheid hadden (**Hoofdstuk 6**). De positief voorspellende waarde van de DST voor psychosociale problemen en slaapproblemen was echter slechts 50% en 30% respectievelijk. Daarom concludeerden wij dat de 'zeer lage dosis' DST geen adequate voorspeller is voor neuropsychologische

bijwerkingen. Het verband tussen gedrags- en slaapproblemen met een hypersensitieve respons in de 'zeer lage dosis' DST, daarentegen, duidt op een mogelijke rol van genetische variatie in het optreden van neuropsychologische bijwerkingen, die variëren tussen patiënten.

Dexamethason dal spiegels waren niet geassocieerd met neuropsychologische bijwerkingen.

Dexamethason kan ook leiden tot significante veranderingen in eetgedrag en energie inname. We hebben prospectief het eetgedrag, de energie en nutriënten inname tijdens dexamethason behandeling bestudeerd (**Hoofdstuk 7**). De energie inname per dag was excessief tijdens een dexamethason blok, en omvatte een toename van energie inname, totaal eiwit, totaal en verzadigd vet, koolhydraten, en zout inname in meer dan 60% van de patiënten. De inname van onverzadigd vet en zout overschreed de gezonde hoeveelheid voor leeftijd en geslacht. Er was een grote variabiliteit in eetgedrag tussen patiënten tijdens dexamethason behandeling, maar het lijngericht eten verminderde significant in de totale groep.

Concluderend suggereren de resultaten van dit proefschrift dat hydrocortison toevoeging een waardevolle behandeling is voor klinisch relevante psychosociale en slaapproblemen door dexamethason in kinderen met ALL. Voordat deze nieuwe behandeling in de kliniek kan worden toegepast, moeten de positieve effecten worden gevalideerd in een grotere groep patiënten met klinisch relevante psychosociale en slaapproblemen.

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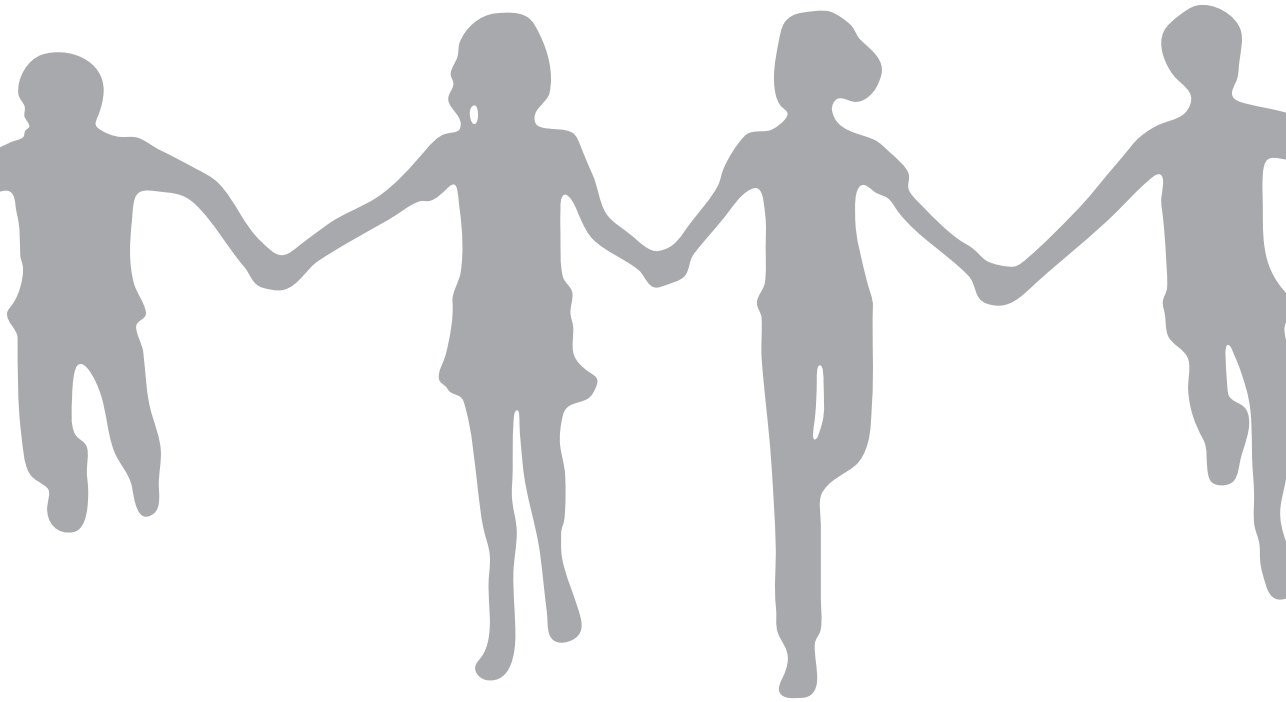
Chapter 10

List of abbreviations



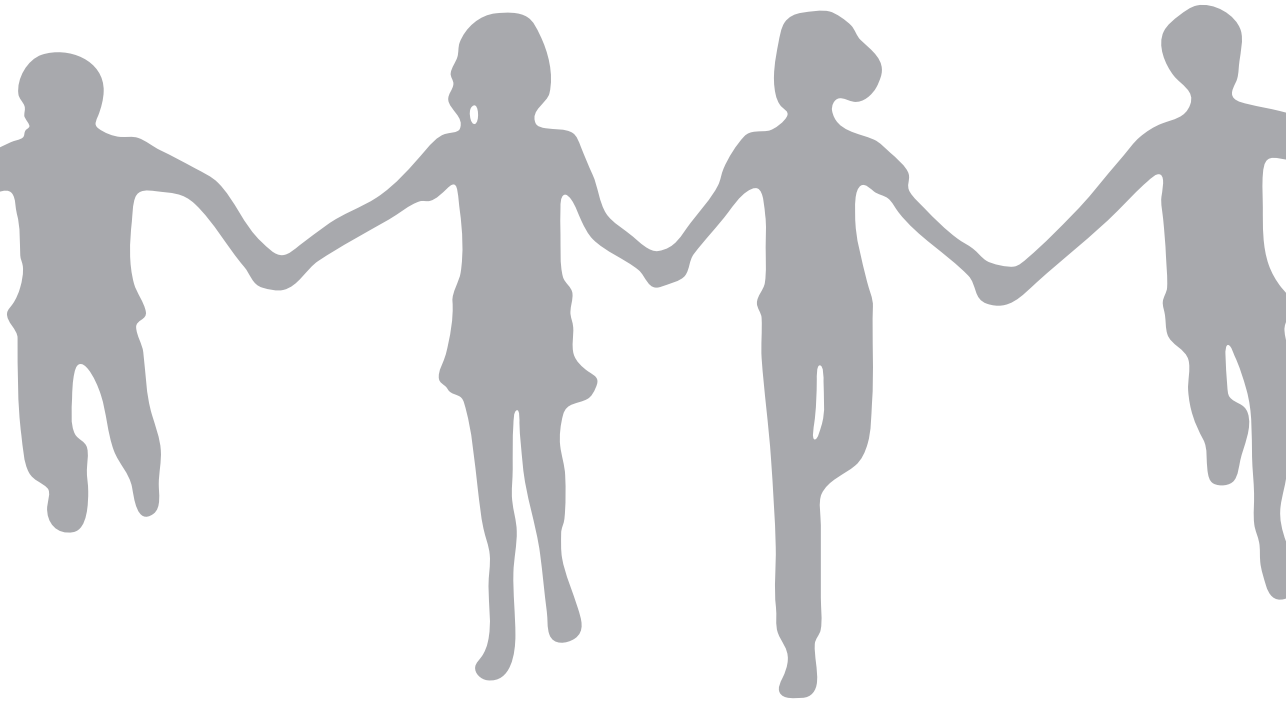
LIST OF ABBREVIATIONS

AA	auditory attention total correct
ANT	Amsterdam neuropsychological tasks program
ALL	acute lymphoblastic leukemia
BASC	behavioral assessment system for children
BMI	body mass index
BPAQ	baecke physical activity questionnaire
BRIEF	behavior rating inventory of executive function
CBCL	child behavior checklist
CTCAE	common terminology criteria for adverse events
DA	disorders of arousal
DC	design copying
DCPT	design copying process total score
DES	disorders of excessive somnolence
DCOG	Dutch childhood oncology group
DEBQ-C	Dutch eating behavior questionnaire for children
DIMS	disorders of initiating and maintain sleep
DSM-5	diagnostic and statistical manual of mental disorders 5th edition
DST	dexamethasone suppression test
GR	glucocorticoid receptor
GRADE	grading of recommendations assessment development and evaluation
HOMA-IR	homeostasis model assessment insulin resistance index
HDL	high-density lipoprotein–cholesterol
HPA	hypothalamus-pituitary-adrenal
IDF	international diabetes federation
IFG	impaired fasting glucose
INE	inhibition total errors
IQR	interquartile range
LDL	low-density lipoprotein-cholesterol
MDT	memory for designs total score
MDDT	memory for designs delayed total score
MetS	metabolic syndrome
MR	mineralocorticoid receptor
MRD	minimal residual disease
MTT	methyl-thiazol-tetrazolium salt drug cytotoxicity assay
NMFC	narrative memory free and cued recall total score
NMRG	narrative memory recognition total score
PCR	polymerase chain reaction

PedsQL	pediatric quality of life inventory
PPV	positive predictive value
PSI	processing speed index
RCT	randomized controlled trial
RDA	recommended daily allowance
RS	response set total correct
RT-qPCR	quantitative real time polymerase chain reaction
SBQ	sleep breathing disorders
SDQ	strengths and difficulties questionnaire
SDS	standard deviation score
SDSC	sleep disturbance scale for children
SHY	sleep hyperhidrosis
SWTD	sleep-wake transition disorders

Chapter 11

About the author



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CURRICULUM VITAE

Lidewij Warris was born on the 3rd of May 1986 in Leiden, The Netherlands. She graduated from secondary school in 2004 at the Stedelijk Gymnasium Leiden. That same year, she started her medical training at the Leiden University. She became interested in oncology, and during her third year she arranged a research elective on 'The role of OATP1B3-mediated transport in cellular sensitivity to anticancer drugs' at the St. Jude Children's Hospital in Memphis, USA (supervisors: dr. A. Sparreboom en prof.dr. A.J. Gelderblom). Inspired by her earlier experiences, she returned in 2010 to Memphis for a clinical elective in pediatric oncology. During the clinical phase of her medical study she was member of the national student board of the Royal Dutch Medical Association (KNMG). After obtaining her medical degree in March 2011, she worked as a pediatric resident (ANIOS) at the LangeLand Ziekenhuis, Zoetermeer. In February 2012, she started her PhD project at the department of pediatric oncology and pediatric endocrinology of the Erasmus MC – Sophia Children's Hospital (supervisors: prof.dr. R. Pieters, prof.dr. M.M. van den Heuvel-Eibrink, dr. E.L.T. van den Akker), which has resulted in this thesis. During this period she was a board member of the Sophia Researchers Association and organized the Theme Sophia Research Days in 2014. In April of this year, she started working as a pediatric resident at the Meander Medical Center in Amersfoort. Lidewij currently lives in Amersfoort, together with Bas Boterman.

LIST OF PUBLICATIONS

- **Warris LT**, van den Heuvel-Eibrink MM, den Hoed MA, Aarsen FK, Pieters R, van den Akker ELT. Does dexamethasone induce more neuropsychological side effects than prednisone in pediatric acute lymphoblastic leukemia? A systematic review. Pediatric Blood Cancer, Pediatr Blood Cancer. 2014 Jul;61(7):1313-1318.

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- **Warris LT**, van den Akker ELT, Bierings MB, van den Bos C, Aarsen FK, Zwaan CM, Tissing WJE, Veening MA, Pieters R, van den Heuvel-Eibrink MM. Eating behavior during dexamethasone treatment in pediatric acute lymphoblastic leukemia patients. Submitted.

PHD PORTFOLIO

Summary of PhD training an teachings activities

Erasmus MC Department:	Pediatric Oncology & Pediatric Endocrinology
Research School:	MolMed
PhD period:	February 2012 – February 2016
Promotors:	Prof.dr. R. Pieters
	Prof.dr. M.M. van den Heuvel-Eibrink
Supervisor:	Dr. E.L.T. van den Akker

1. PhD training

	Year	ECTS
General courses		
Pubmed and Endnote, Medical Library, Erasmus MC	2012	0.3
Integrity in scientific research, Erasmus MC	2012	2
Good clinical practice, Erasmus MC	2012	1
CPO mini symposium	2013	0.3
Research management	2013	1
Biomedical English Writing	2014	2
Specific Courses		
Basic and Translational Oncology, MolMed, Erasmus MC	2013	1.8
Masterclass medical Business, VUMC	2013	2
Seminars and workshops		
Pediatric research days, Erasmus MC	2013-2015	0.9
Young investigator days, TULIPS/ NVK	2013-2015	0.9
Research Retraite, SKION-Princess Máxima Center	2013-2015	0.6
Annual KiKa PhD day	2013-2015	0.9
Annual PhD day, Erasmus MC	2012-2014	0.6
Weekly Pediatric Oncology meetings, Erasmus MC	2012-2015	2
Weekly Quality of Care and Toxicity meetings, Erasmus MC	2012-2015	2
Weekly Pediatric Endocrinology meetings, Erasmus MC	2012-2014	1.5
Weekly Pediatric Oncology research meetings, Princess Máxima Center	2015-2016	1
AAV Wetenschapsmiddag	2013-2015	0.3
Conferences		
International		
9th Biennial Childhood Leukemia Symposium, Prague (poster presentation)	2014	1
46th Congress of the International Society of Pediatric	2014	1

Oncology (SIOP), Toronto (oral and poster presentation)		
47th Congress of the SIOP, Cape Town	2015	1
American Society of Hematology Annual Meeting, Orlando (poster presentation)	2015	1
Endocrine Society Annual Meeting, Boston (3 poster presentations)	2016	1
10th Biennial Childhood Leukemia Symposium, Athens (4 poster presentations)	2016	1
48th Congress of the SIOP, Dublin (oral and poster presentation)	2016	1

National

KiKa Site Visit, Amsterdam (oral presentation)	2014	1
AAV Wetenschapsmiddag (oral presentation)	2015	1
Pharmakids meeting Erasmus MC (oral presentation)	2016	1

2.Teaching activities

Intern teaching: introduction to pediatrics (ICK)	2012-2015	2
Asssistant at weekend school Child and Growth	2013	0.3
Supervising minor students Pediatric Oncology	2013	2

3.Other activities

Board member of Sophia Researchers Association	2013-2015	2
PhD day organizing committee, Erasmus MC	2014	1
Sophia Research Days organizing committee, Erasmus MC	2014	1
SIOP Young Investigators Network committee	2015-2016	1
Peer review of articles for international scientific journals	2014-2015	0.5

4. Awards

Abstract Achievement Award, ASH Orlando	2015
SIOP Young Investigator Award, Dublin	2016

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Lidewij